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State of the Art

Deposition of Aerosol in the Respiratory Tract[1-3]

JOSEPH D. BRAIN and PETER A. VALBERG

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Introduction

Depending on their size and activity, adult humans breathe 10,000 to 20,000 L of air daily. Contaminating particles enter the body in this volume of air and are potentially hazardous. Most pulmonary disease is initiated, or at least aggravated, by the inhalation of particles. Exposure to myriad micro-organisms, smokes, dusts,

allergens, and other toxic aerosols is a feature of everyday life. The sizes of some commonly encountered particles are given in figure 1. Moreover, the same thinness, delicacy, and vast surface area that make the air-blood barrier ideal for the rapid exchange of O_2 and CO_2, decrease its effectiveness as a barrier to deposited particles.

Not only do inhaled particles cause pulmonary disease in humans, but they may also be used to produce pulmonary disease in animals experimentally, to treat disease, or to alter physiologic states in experimental or clinical tests. In each instance, a detailed description of the pattern of aerosol retention is useful; frequently it is essential. This review focuses on deposition of inhaled aerosols. It discusses the major classes of inhaled particles and their significance to human health; it describes mechanisms of deposition and discusses factors that determine the effectiveness of these mechanisms. It describes how to generate aerosols, how to characterize exposures to aerosols, and how to measure particle retention. Although gases usually accompany particles and gas-particle interactions are frequently important, the effects and uptake of inhaled pollutant gases will not be covered. Nor will this paper be all inclusive with regard to aerosols. The problem of accurately describing the deposition of aerosols in mammalian lungs has captured the energy and imagination of many investigators. Published symposia such as the First (Oxford), Second (Cambridge), Third (London), and Fourth (Edinburgh) International Symposia on Inhaled Particles (1–4), serve as excellent sources of information in this area. In addition, several papers and books reviewing deposition and clearance processes are available (5–11). Much of this work has been made possible by major advances in aerosol science and technology. Recognition of the importance of characterizing aerosols has increased along with our ability to generate and measure them accurately.

Despite these advances and an abundance of some kinds of data, many features of aerosol deposition have not been adequately investigated. For example, few studies relate changes in lung volume and breathing pattern to the spatial pattern of aerosol deposition. More generally, quantitative data describing actual sites of particle deposition are inadequate. For example, although the International Committee on Radiation Protection (ICRP) Task Group Report lung model (8, 12) is widely known and used, it has never been tested directly. Conceptually, it is easy to divide the lung into ciliated conducting airways and nonciliated alveolar regions, but it is physically difficult to separate and measure their aerosol retention indepen-

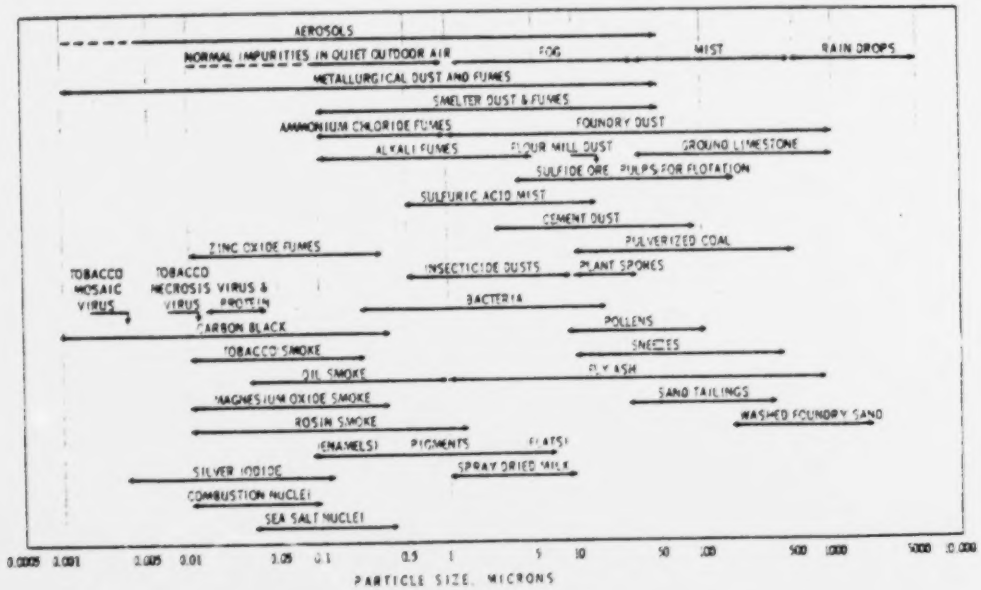


Fig. 1. Types and sizes of some commonly encountered aerosols.

dently. Another major problem is how to calculate the dose to the respiratory tract when the retained aerosol is not distributed uniformly. Should the dose be averaged over the whole lung, or should the local airway or alveolar epithelial dose be estimated? An additional problem is that, although a variety of species have been used for aerosol studies, no adequate overview of species differences exists. We expect that aerosols will be used increasingly as probes of pulmonary function, for experimental studies of pathology, and for therapeutic purposes. These applications depend on the ability of the investigator to introduce known amounts of aerosol into animals or humans. The goal may be to measure airspace dimensions (13, 14), to simulate an urban or occupational exposure of humans, to deliver to animals a disease-producing dose to describe the pathogenesis of a lesion, or to evaluate a prophylactic or therapeutic strategy. In every instance, describing the deposition and retention of the aerosol is essential.

The terms "deposition" and "retention" are not synonymous. The actual amount of aerosol found in the lungs at any time is called the retention. The retention is determined by deposition and clearance. Deposition refers to the initial processes that determine what fraction of the particles in the inspired air are caught in the lungs and fail to exit with the expired air. The output of particles previously deposited in the lungs is called "clearance" and refers to the processes that physically expel the particles from the lungs. The relative rates of deposition and clearance thus determine the equilibrium concentrations of particles in the lungs. Only immediately after an infinitely short exposure is retention equal to deposition.

When the exposure is continuous, the equilibrium concentration (achieved when the clearance rate matches the deposition rate) is also the retention. Thus, the relative rate constants of deposition and clearance together determine the equilibrium concentration. It is the equilibrium concentration, or retention integrated over time, and the properties of the particle that are presumably related to the magnitude of a pharmacologic, physiologic, or pathologic response. Clearance mechanisms include solubilization and absorption, sneeze, cough, mucociliary transport, and alveolar clearance mechanisms involving pulmonary macrophages and other mechanisms. These factors work together to keep the respiratory tract relatively free of foreign material. Even the diseased and blackened lungs of miners who die of coal worker's pneumoconiosis contain less than 2 % of the dust originally deposited there. The healthy lung exhibits even greater potency in the pursuit of cleanliness. Although these clearance mechanisms are of considerable importance and must be taken into account in estimating retention, they are beyond the scope of this review. Excellent summaries exist (1–4, 7, 15–18). It should be kept in mind that clearance is often of greater significance than deposition. For example, if the clearance efficiency of a particle decreases from 99 to 98 %, retention doubles. Thus, a 1 % change in total clearance produces a 100 % change in retention. Clearance efficiency may be the determining factor for total integrated exposure and, consequently, the probability of a pathologic or physiologic response.

Major Classes of Inhaled Particles And Their Significance

Infectious Particles

Liquid droplets containing a variety of organisms are produced during coughing, sneezing, and talking, and can be an essential vector for infection. Their initial mean size is 3 to 20 μm (19, 20); they have a fast settling rate, and if inhaled, are deposited in the nose or upper respiratory tract. However, Wells (21) has pointed out that these liquid droplets evaporate rapidly and are quickly transformed into much smaller particles containing the residue of the original liquid. Wells (21) called these residual particles "droplet nuclei" and showed that they have small diameters (a few micrometers or less) and thus can reach the smaller airways and alveoli. Droplet nuclei may contain virulent pathogens, which can remain viable for hours or days. Because the settling rates of the droplet nuclei are low, they may remain suspended in air for a long time.

Infectious aerosols containing viable bacteria or viruses may also be produced by the resuspension of particles from clothing, furniture, sputum, or feces. Epidermal bacteria may also appear on shed skin flakes and hair. Natural processes such as wind, waves, and other mechanisms for producing water droplets may also create aerosols containing pathogenic organisms. Other man-made sources include air conditioners, sewage treatment plants, and industrial processes involving animals or animal byproducts. Edmonds (22) has discussed the airborne patho-

gens, their modes of production, and their potential for causing disease.

A number of epidemiologic studies implicate such aerosols in the transmission of tuberculosis, measles, influenza, smallpox, and other diseases (23). McDermott (24) edited a series of papers given at a conference on airborne infection that provided evidence for the airborne transmission of plague, brucellosis, *Staphylococcus*, *Streptococcus*, mycoses, anthrax, Q fever, influenza, and histoplasmosis. In his book on communicable diseases, Top (25) organized the diseases according to the most common portal of entry. The largest class of diseases includes those that enter the body through the respiratory tract. In addition to the diseases already mentioned, Top (25) lists diphtheria, meningitis, leprosy, chicken pox, lymphocytic choriomeningitis, pertussis, and rubella.

Transmission of human disease by aerosols has been increased by urbanization, because airborne pathogens are especially common in indoor spaces. The costs of disease spread by the aerosol route are vast. The influenza epidemic of 1918 to 1919 resulted in more than 500,000 deaths in the United States alone and more than 20 million deaths throughout the world (22).

The significance of airborne infection extends from the home to schools, office buildings, and hospitals. There is growing appreciation that nosocomial (hospital-acquired) infections are a major medical problem. The Center for Disease Control in Atlanta has reported that significant hospital-acquired infection will be diagnosed in 3 to 6 % of all hospitalized patients (26). Many of these infections will be of the respiratory tract. Such infections are likely because of the number and virulence of organisms in the environment and the fact that normal host defenses may be suppressed by disease or drug treatment. On some occasions, procedures (e.g., bronchoscopy) and equipment (e.g., inhalation therapy) may contribute directly to respiratory tract infections. Problems caused by room air vents, air conditioner coils, or cooling towers colonized by various pathogens have also been reported; Legionnaires' disease is a recent example. Endotracheal intubation may also contribute to the problem, because the normal defense mechanisms of the upper respiratory tract are bypassed. Leedom and Loosli (27) have recently reviewed the importance of airborne pathogens in the indoor environment with special reference to nosocomial infections.

Growing evidence suggests that therapeutic aerosol generators and nebulizers may sometimes be contaminated and serve as a source of nosocomial infection. Scrupulous attention should be given to keeping these devices clean and sterile. *Pseudomonas*, *Proteus*, *Alcaligenes*, *Herellea*, *Flavobacterium*, and other organisms have been identified in the reservoirs of nebulizers used to administer moisture with O_2-enriched air (28, 29). Precautions must be taken to eliminate bacterial colonization of these devices. Aerosol generators and all associated tubing should be thoroughly cleaned, soaked in germicidal solutions, or gas sterilized. The increasing use of disposable components also helps minimize infection.

Allergens

Airborne allergens cause rhinitis and other forms of chronic or recurrent nasal difficulties, hay fever, asthma, hypersensitivity pneumonitis, and other allergic diseases. These allergens originate from a variety of animal and plant sources, including pollens, spores, and other organic dusts. They may include airborne tree pollens, such as those from elms, maples, ashes, oaks, and walnuts. Almost all grasses are wind pollinated and may be major causes. The book, *Aerobiology*, edited by Edmonds (21), provides a thorough review of types of pollen, their modes of distribution, and their pathogenic effects. Muir (30) has also reviewed the sources and effects of airborne allergens. Fungal spores, spores of slime mold, fragments of soil algae, bacterial products, and insect debris may all be offenders. Animal dander from living quadrupeds and from animal products such as wool or feathers are additional sources. Many of these agents are complex; ragweed pollen, for example, contains many different allergenically active components.

In addition to natural sources, human activity may produce significant quantities of allergenic particles. These include some simple chemicals (platinum salts and tannic acid), insect parts, animal dander, and plant materials used in industrial processes, such as cottonseed oil or castor bean allergen (31, 32).

The most commonly encountered aero-allergens are ragweed pollen, mold spores, and house dust (33). Hay fever is estimated to affect approximately 5 to 10 % of the U.S. population. During ragweed season, daily pollen concentrations in most of the eastern and central United States commonly reach 250 to 1,000 pollen grains/m_3 of air, with some as high as 4,000

grains/m³. Durham (34) estimated that approximately 275,000 tons of ragweed pollen are released into the air each year in the United States. Most airborne tree pollen is shed during spring and early summer, whereas grass pollen is shed in mid-summer; weed pollen, in late summer and fall (35). There are wide seasonal and regional differences, with the highest counts found in Kansas; the lowest counts, in the states of Washington and Oregon. Local vegetation, pollen size, wind direction and speed, and rainfall are major factors.

Most pollen particles are large, ranging from 20 to 60 μm in diameter; mold spores and house dust are smaller (33). Because of their large diameters, most pollen grains are deposited in the upper respiratory tract, except during mouth breathing. Even though most pollens do not penetrate deep into the lung, because deposition is a probabilistic event, large particles or fibers are occasionally deposited on surfaces of small airways and alveoli. Hobday and Townley (36) identified whole ragweed pollen grains as far as the intrapulmonary bronchi in guinea pigs. Relatively few intact pollens penetrate to alveoli. Sometimes, fragments of pollen particles may be within the respirable range. Busse and associates (37) reported that a significant fraction of the antigenicity of pollen suspended in the atmosphere was found in small (< 5-μm) fragments of pollen, rather than in whole particles.

Occupational Dusts

In addition to the combustion products characteristic of any energy-consuming, industrialized society, there are aerosols that are unique to specific industrial processes. These particles present special hazards to plant employees and to the populations living nearby. Mines, cement mills, iron and steel mills, oil-processing plants, and factories of every kind often produce particles as an unwanted accompaniment to their primary product. Dusts containing silica, asbestos, metallic fumes, and organic matter are inhaled every day by millions of workers.

TABLE 1

IMPORTANT AEROSOLS PRODUCING OCCUPATIONAL LUNG DISEASES

Agent	Examples
Organic	
Natural	
Fossil	Coal, carbon black, graphite, charcoal
Microbial	
Bacterial	Tuberculosis (hospital workers), anthrax (wood workers), *Bacillus subtilis* enzyme (detergents)
Fungal	Coccidioidomycosis (farm workers), histoplasmosis and cryptococcosis (aviary workers, bird fanciers), thermophilic actinomycetes
Viral/Rickettsial	Psittacosis (pet shop workers), Variola and Q fever (lab workers)
Vegetable	Moldy hay ("farmer's lung"), mushroom compost, bagasse (sugar cane), maple bark, malt, grain weevil, cork, roof thatch, cotton, flax, hemp
Animal	Pigeon, parrot, and hen droppings; pituitary snuff
Synthetic	
Plastics	Polytetrafluoroethylene, toluene diisocyanate
Reagents	Isopropyl oil, organic solvents
Inorganic	
Free silica	
Crystalline	Quartz, tridymite cristobalite
Amorphous	Diatomaceous earth, silica gel
Silicates	
Fibrous	Asbestos, sillimanite, talc
Other	Mica, Kaolin, cement dust
Metals	
"Inert"	Iron, barium, titanium, tin
Other	Aluminum, beryllium
Respiratory carcinogens	Arsenic, cobalt, nickel, hematite, uranium, asbestos, chromates

The respiratory tract is the most important route for the entry of toxic substances from occupational exposures; some of the most significant agents are summarized in table 1. Lanza (38) reviewed the relationship between chronic respiratory disease and inhaled silica, asbestos, diatomaceous earth, and beryllium. The long-term respiratory effects of these substances persist and may even grow worse after the exposure has stopped. Silicosis remains the most severe and rapidly fatal form of pneumoconiosis encountered in the United States.

Byssinosis is another pulmonary disease caused by particulates. The bract from cotton induces release of histamine from airways, resulting in bronchoconstriction and dyspnea. Occupational asthmas and other allergic diseases of the lung are caused by agents ranging from simple chemicals to complex animal proteins.

Many industrial substances also cause lung cancers. Eckardt (39) estimated that 10 % of all occupational cancers involve the respiratory tract. Chromate, nickel compounds, and asbestos may all cause lung cancer. Uranium miners have excessive rates of lung cancer, as will be discussed in the next section.

Radioactive Particles

Natural processes, weapons testing, and the nuclear industry contribute to the radioactive content of the air. Radioactive daughters of radon produced from naturally occurring radium can be inhaled (40). The detonation of nuclear devices in the atmosphere has released large quantities of fission products into the atmosphere. The high neutron fluxes associated with exploding nuclear weapons can also activate pre-existing or blast-created dusts. The increasing numbers of nuclear reactors and problems of waste disposal and fuel reprocessing may contribute to radioactivity in the air through routine operation and infrequent accidents. Mining, milling, and fuel fabrication can give rise to radioactive metallic oxides. Any laboratory or industrial process involving radioisotopes may provide additional sources of radioactive particles. An increasing number of space satellites now contain radioactive material that may be released through re-entry, burn-up, or unsuccessful launch (41).

For the general public and for uranium miners, radon daughters are the most significant naturally occurring inhaled radionuclides. Radium, present in the earth's crust, decays to radon, which escapes as a gas and then decays to radioactive polonium, lead, and bismuth. The polonium isotopes emit alpha radiation and account for the major portion of the dose to the human respiratory tract. Some of these radon daughters may be in the form of "free ions" and have a collection efficiency in the respiratory tract approaching 100 %. Others attach to dust particles, so that the size distribution of radioactivity reflects that of the dust in the mine or ambient atmosphere. The abnormally high incidence of cancer of the respiratory tract among uranium miners in both the United States and Europe has been attributed to these radon daughters (40, 42–44). A 29-fold increased risk of lung cancer among fluorspar miners in Newfoundland has also been attributed to the inhalation of excessive concentrations of radon and its daughter products (45). Fly ash from coal-burning power plants also contains small amounts of radioactivity, principally radium-226 and radium-228. Eisenbud and Petrow (46) estimated that conventional fossil fuel plants discharge relatively greater quantities of radioactive materials into the atmosphere than do nuclear power plants of comparable size.

The quantites of airborne radioactive material are under constant surveillance. Bradshaw and Setter (47) found an average of .5 pCi of respirable particles/m^3 of air. The particles were collected by a cyclone separator that simulated the capture of particles by the respiratory tract. Lockhart and associates (48) described the size distribution of radioactive aerosols in the atmosphere in the absence of significant air pollution. They showed that short-lived radon daughter products such as lead-214 and bismuth-214 are present; these are associated primarily with particles less than 0.3 μm in diameter. Fission products recovered from the stratosphere are associated with particles of average diameter 0.5 to 1.0 μm.

Much of the airborne radioactivity is in the form of insoluble particles, which are cleared from the lungs more slowly. Scalf and Ledbetter (49) measured the solubility of radioactive dust sampled on membrane filters. They found that the percentage that is soluble depends on meteorologic conditions and is pH sensitive. Only 60 % of the particles were soluble at a pH of 7, the pH of minimal solubility. Mamuro and coworkers (50) found that approximately 50 % of the total activity from dust samplers was insoluble in rain or distilled water. Among fallout particles not immediately soluble in rain water, less than 24 % of the activity could be dissolved

away during a 24-h leaching with 6 *N* hydrochloric acid.

Atmospheric Dusts

This broad classification includes resuspended dirt, products of combustion processes, and naturally occurring biologic aerosols. Wind and vehicular traffic constantly resuspend soil, debris, and pollen. Natural sources include resuspension of soil and rock debris by wind, forest fires, sea salt, and volcanic debris (51). A recent Subcommittee of the National Research Council has discussed the sources, characteristics, and behavior of naturally occurring aerosols in the atmosphere (52). Soot containing small particles of carbon and other organic compounds is constantly being formed from the burning of fossil fuel. Gas phase reactions activated by sunlight also generate new particles through chemical changes involving man-made pollution (51). Man-made dusts from transportation, industrial processes, and incineration are quantitatively less significant than naturally occurring dusts; they account for only 185 to 415×10^6 metric tons/yr of an estimated total annual amount of atmospheric dust of 985 to $2,615 \times 10^6$ metric tons. However, man-made sources may be more important in terms of per capita exposure and in terms of their biologic significance. They are highly localized on less than 1 % of the earth's surface and in areas of the highest population density (51, 53).

In intense smog, concentrations of 10^{11} particles/m^3 may be reached; even nonpolluted air has at least 1 % of that amount. Typically, cities with populations of 400,000 to 700,000 have dust concentrations averaging 129 $\mu g/m^3$ of air. If an individual's ventilation is 20,000 L/day, then approximately 2.5 mg of dust is inspired each day. The dust concentrations of cities with populations exceeding 3 million are an average of 50 % higher (54). During conditions of serious smog, concentrations may exceed 5,000 $\mu g/m^3$ of air.

Because man spends much of his life indoors, it is essential to quantify the levels of pollutants there as well as outdoors. Energy conservation has led to an increased tendency to seal buildings, which has increased the importance of interior pollution. Evidence now suggests that the indoor concentrations of pollutants may be very different from those outside, depending on the location of the source, the nature of the pollutants, and interior and exterior barriers. The contribution of tobacco smoke to indoor air pollution is now considered to be important. In 1978, a symposium was held in Copenhagen to discuss indoor climate (55).

Particle size is important, because it determines the persistence of the aerosol. Both size and concentration decrease with increasing altitude or distance from urban centers or other sources of aerosols. Willecke and Whitby (56) have described the size distribution of ambient aerosols in urban environments in the United States. More recently, Fennelly (57) has reviewed the significant sources of man-made particulate pollution in the United States, the mechanisms of formation of the particles, and their deleterious effects. Atmospheric particles show a bimodal distribution. One peak between 5 and 10 μm reflects primary particles formed by a variety of physical and chemical means, such as wind-dispersed soil dust and solid industrial emissions released into the air. A smaller peak between 0.2 and 0.5 μm represents secondary particles that are probably the products of chemical reactions taking place in the atmosphere (57). Although smaller particles may contribute less than larger ones to the total weight of particles, there are more smaller particles and they have the largest surface area. Their elemental composition may also differ considerably. Small size increases the probability of penetration into small airways and alveoli, and the large surface area favors gas adsorption and interaction with the particles (58).

The human health effects of atmospheric particles can be considerable. First, there may be temporary episodes of severe air pollution that cause a transient increase in morbidity and mortality. These incidents are produced when a temperature inversion creates a layer of still air that gradually accumulates combustion products and other pollutants. These severe episodes of air pollution result in deaths and severe symptoms primarily among persons with pre-existing cardiac or pulmonary diseases. Perhaps the 2 most serious of these episodes occurred in Donora, Pa. (59), during which 17 deaths and symptoms in almost one half of the population were reported, and in London (60), when 4,000 excess deaths were recorded in a single week.

Secondly, even in the absence of acute air pollution, there may be effects of long-term, low-level pollution on large segments of the population. Several studies suggest that general air pollution in large industrial cities can cause increases in pulmonary disease. Dean (61), for example, found that the rate of lung cancer among per-

sons who had migrated to urban areas of Great Britain from South Africa was higher than that among persons who remained in South Africa. Reid (62) also showed that the mortality rate among persons with chronic bronchitis is higher in urban than in rural areas. These studies suggest that exposure to polluted air may be significant: the fraction of the observed effect that is actually caused by particles in polluted air has not been determined. Doerner (63) and Ferris (64) have summarized the correlations between air pollution and chronic pulmonary disease. Lave and Seskin (65) found significant correlations for respiratory disease, cardiovascular disease, and cancer with such indices as dustfall, sulfation rate, and concentration of suspended particles.

Consumer Aerosol Products

The common components for all spray can aerosols are a propellant, a solvent, and an active ingredient. Nonmedical aerosol preparations include antiperspirants, hairsprays, insecticides, and other household and industrial products. Until recently, the most frequently used propellants (gases that provide the force to disperse the active ingredient and solvent) were fluorocarbons. Once considered to be nontoxic, they are now generally accepted as hazardous, particularly to the heart (62). There has also been considerable concern about the possible decrease in the protective ozone layer in the stratosphere due to overuse of fluorocarbons. In 1975, the annual per capita use of pressurized products in the United States reached approximately 15 cans, but the rate has probably decreased since then.

The particular active ingredient and solvent included in an aerosol product differentiate among myriad uses and exposure conditions for the user. A survey of the particle characteristics of frequently aerosolized products ranging from antiperspirants to spray paints revealed mass median aerodynamic diameters of 2.4 to 5.6 μm and concentrations of several mg to approximately 1 g/m^3 in the "breathing zone" (66). Ninety per cent or more of the weight of these aerosols can probably be accounted for by particles larger than several μm in diameter (67). Once inhaled, these particles are deposited principally in the upper airways, whereas the submicrometric particles comprising a smaller portion of the total mass are deposited principally in the small airways and alveolar regions. The submicrometric particles are presumed to be responsible for the granulomatous lesions of the lungs found in hairdressers (68), and for the reversible narrowing of small airways observed immediately after exposure to hair spray (69). Even these effects are controversial, and the health impact of the thousands of aerosol products is unknown because of the intermittent and varied conditions of use and the associated expense and difficulty of carrying out definitive epidemiologic studies. In some instances, no animal studies are performed before new and potentially hazardous aerosol products are introduced into the marketplace.

Cigarette Smoke

An aerosol of particular importance to human health is tobacco smoke, which may contain as many as several billion particles/cm^3 (70). Most smoke particles are 0.2 to 0.6 μm in diameter, with essentially none larger than 1 μm in diameter. Such small particles penetrate deep into the lung during inhalation. The size of smoke particles depends on the concentration of the smoke, because agglomeration is likely. Hinds (71) reported that the mass median aerodynamic diameter of smoke decreased from 0.52 to 0.38 μm as the dilution of the mainstream smoke was increased from 10:1 to 700:1. Approximately 50 to 80 % of inhaled smoke is deposited in the lungs, resulting in the deposition of approximately 25 mg of particulate matter per unfiltered cigarette (72). In 1 yr, a typical smoker of 1 pack of cigarettes/day inhales 50,000 to 70,000 puffs of cigarette smoke and deposits approximately 180 g of particulate matter in the lungs (73).

Certain constituents of cigarette smoke have been classified as probable contributors to the health hazard of smoking. They include tar, nicotine, phenolic compounds, chlorophenothane (DDT), nickel compounds, and others (74). In addition, several potential carcinogenic agents are contained in smoke, including radioactive particles such as polonium-210 (75, 76), metallic constituents such as nickel and cadmium (77, 78), aromatic amines (79), and aromatic hydrocarbons (80). Falk (81) has recently reviewed the composition of tobacco smoke.

In 1964, the first Surgeon General's Report on Smoking and Health (82) summarized the evidence that tobacco smoke has deleterious effects on health. It convincingly showed a relationship between inhaled cigarette smoke and lung cancer as well as cancer at other sites,

TABLE 2

CLINICAL USES OF AEROSOLS

Uses	References*
Diagnostic	
Agents for provocation tests to assess airway responsiveness	
Drugs	Spector and Farr (83) Lockey and Bukantz (84)
Specific allergens	Spector and Farr (85)
Estimation of extent of gas mixing	Altshuler, et al. (86, 87) Heyder and Davies (88) Muir (89)
Estimation of sizes of air spaces	Palmes *et al.* (14, 90, 91)
Radioactive and radiopaque aerosols to study deposition and clearance of particles	Albert and Arnett (16) Nadel *et al.* (92)
Radioactive aerosols for assessment of regional ventilation	Taplin *et al.* (93)
Tantalum powder as a contrast medium for bronchography	Nadel *et al.* (94)
Therapeutic aerosols commonly used	
Bronchodilator drugs	Jenne (95)
Sympathomimetic drugs	Innes and Nickerson (96)
Xanthines	Stewart and Block (97)
Anticholinergic drugs	Klock (98)
Other drugs for treatment of symptoms of asthma	
Disodium cromoglycate to decrease incidence and severity of allergic asthmatic attacks	Altounyan (99) Irani et al. (100)
Corticosteroids to suppress inflammation	Campbell (101)
Induction of oropharyngeal, laryngeal, or tracheobronchial anesthesia	Christofiridis *et al.* (102) Wanner *et al.* (103)
Water or saline aerosol for humidification	Egan (104) Wolfsdorf *et al.* (105)
Potential uses in various stages of investigation and deployment.	
Agents for altering rheologic properties of mucous secretions to aid in clearance	
Detergents	Walkenhorst and Dautrebande (106)
Mucolytic agents	Barton (107)
Proteolytic agents	Limber *et al.* (108)
Antimicrobial agents	
Antibacterial	Feeley *et al.* (109)
Antifungal	Kilburn (110)
Antiviral	Scott and Sydiskis (111)
Regional cancer chemotherapy	Heyes and Catherall (112)
Treatment of alveolar instability with dipalmitoyl lecithin (e.g., in neonates)	Merrill *et al.* (113)
Immunization of man and animals against bacterial and viral pathogens	Hitchner and Reisig (114) Middlebrook (115) Waldman *et al.* (116)

* These references are not inclusive or complete; rather, they are examples chosen from the voluminous literature.

function associated with humidity therapy (123, 124).

The usefulness of mucolytic agents has not been adequately demonstrated. It is clear that drugs contained in these aerosols alter the characteristics of mucus *in vitro* and probably *in vivo*. However, it is not clear whether these alterations in rheologic properties are appropriate and whether they actually improve mucociliary transport, cough, and other clearance mechanisms.

There are other more generic problems. In most cases, it is difficult to estimate or calculate the dose of a drug given to a patient by aerosol. Although a large number of manufacturers make a variety of aerosol generators and nebulizers, many fail to provide adequate information regarding the size distribution of the aerosols produced. Thus, even when armed with an adequate knowledge of principles of aerosol deposition, the conscientious scientist or clinician will still have a difficult time estimating the extent or site of aerosol deposition. Some devices produce small particles in the range of 0.5 to 2

chronic bronchitis, pulmonary emphysema, and cardiovascular disease (82). Not only is cigarette smoke toxic, but it also acts synergistically with other toxic aerosols and may retard their clearance (81). Since 1964, the prevalence of regular cigarette smoking in the adult population has decreased from approximately 42 to 33 %; yet in 1978, an estimated 54 million men and women smoked 615 billion cigarettes (73). The extent of health effects related to cigarette smoking is underscored by its existence as the largest preventable cause of death in the United States (73).

Clinical Aerosols

Not all aerosols are harmful. Dozens of pharmaceutical companies manufacture and promote aerosols that are purported to relieve bronchospasm, congestion, edema, allergy, and inflammation; to decrease the viscosity of mucus; to treat sinus infection, and so on. The aerosols are commonly delivered with a Freon-powered hand-held nebulizer. More complex and expensive nebulizers are driven by compressed air or use ultrasonic energy.

There are reasons why aerosol administration of drugs may be preferred. Using drugs given by the pulmonary route, it should be possible to achieve high therapeutic concentrations of drugs locally or topically within the respiratory tract without unwanted high systemic concentrations and resultant side effects. For example, the use of bronchodilators seems to be on firm footing. It is widely accepted that a variety of drugs given in aerosol form can relieve the symptoms of asthma both subjectively and objectively. Significant, reproducible changes occur in pulmonary function tests after their use.

The respiratory tract also provides a convenient and rapid route of access to the bloodstream for some soluble or easily absorbed drugs; thus it offers a way to achieve therapeutic systemic concentrations as well. Some enzymes and drugs that ordinarily have no access to respiratory surfaces because of their size or charge can also be conveniently administered by aerosol.

Aerosols can also be used to obtain elusive information about pulmonary function, such as the speed of mucociliary transport or the extent of gas mixing. A summary of some of the clinical uses of aerosols is shown in table 2. Aerosols may also be useful for provocation testing. There is considerable interest in the distribution of airway responsiveness in animal and human populations as it relates to the diagnosis and treatment of asthma. Frequently, skin tests have little relation to airway reactivity, and provocation tests are useful with such agents as methacholine. Histamine (85) can be used, although considerable care should be taken not to compromise pulmonary function too severely. In addition, Palmes and co-workers (90, 117) have suggested that the extent of aerosol deposition during breath holding after inhalation of a single breath of aerosol serves as a tool for estimating the size of air spaces in human lungs. They demonstrated that the aerosol in expired air decays exponentially with the breath-holding time and that the decay constant varies as a function of lung volume and pulmonary disease.

Inhalation of aerosols has become an established component of respiratory therapy and is discussed in most major texts (104). However, many questions remain regarding the indications for and the effectiveness of clinical aerosols. Among other topics, the effectiveness of clinical aerosols used by outpatients was discussed at the Conference on the Scientific Basis of Respiratory Therapy held in 1974 (118). In November 1979, another conference focused on the Scientific Basis of Respiratory Therapy of In-Hospital Respiratory Therapy. In addition, a previous State of the Art article discussed how aerosols of drugs effective against bronchospasm and the dissolution of secretions can be used in the treatment of chronic obstructive pulmonary disease (119). Miller (120) has also reviewed the use of aerosol therapy in the treatment of acute and chronic respiratory disease.

A major concern about aerosol therapy is whether the aerosol reaches the desired location, because aerosol size and patient use are not always adequately controlled. For example, there is evidence that mist tent therapy fails to deposit a significant number of fluid droplets in the airways and alveoli. In one study, 6 healthy adults and 8 patients with cystic fibrosis received a mixture of 10 % glycol in water generated with an ultrasonic nebulizer and tagged with technetium-99m (^{99m}Tc). Only 3 % of the activity nebulized actually entered the body. Of this, 90 % of the inhaled radioactivity initially lodged in the nasopharynx and rapidly appeared in the stomach; very little activity was detected over the lungs (121). Asmundsson and co-workers (122) also showed that a relatively small fraction of the saline aerosol generated is deposited in the lungs. Clinical studies have also failed to show objective changes in pulmonary

μm, which are ideal for delivering drugs to airways and alveoli. On the other hand, atomizers create large particles that are deposited primarily in the oropharynx. Not all nebulizers give an even dose, and many patients have difficulty in releasing the proper dose at the correct time. Many devices require intelligent use by the patient and are frequently misused. Patient abuse and failure to follow prescribed schedules are also a problem. Little is known about the distribution and metabolism of the drugs given. Comprehensive studies describing the accumulation of drugs in blood or urine, or the site of action within the respiratory tract, are seldom available. Normally, the usefulness of each aerosol is justified on the basis of its ability to alter symptoms and signs. Another inherent problem is that stiffened or obstructed parts of the lung will get little or no drug. Frequently, it is the diseased or damaged portions of the lung that are most in need of therapeutic intervention. Unfortunately, the nonventilated areas will not directly receive any of the aerosolized drugs.

Other hazards that are now more widely recognized are related to the cardiopulmonary toxicity of the propellants used for bronchodilator aerosols (125, 126). Freon propellants may be fatal because of their role in acute heart failure due to arrythmias caused by sensitization of the myocardium to circulating catecholamines (127).

Mechanisms of Deposition

The respiratory system acts like a filter in that a significant fraction of the aerosol present in the inspired air is removed during its movement into and out of the lungs. Several separate mechanisms are active in this "filtering process." These are shown schematically in figure 2 and are described later. More detailed treatments have been given by Morrow (128) and by Mercer (129). Comprehensive treatises on the behavior of aerosols are also available (130, 131).

Sedimentation

All particles with density (ρ_{part}) greater than that of air (ρ_{air}) experience a downward force due to gravity. The magnitude of this force (F_{grav}) is calculated as follows:

$$F_{grav} = V_{part}(\varrho_{part} - \varrho_{air})g \qquad [1]$$

where V_{part} is the volume of the particle and g is gravitational acceleration. A particle accelerates downward until its velocity increases to the point where the retarding force due to its motion through air just balances its weight. If the particle is spheric, and if it is small enough that viscous forces are the primary resistive forces, then Stokes' Law can be used to predict the retarding forces:

$$F_{resist} = 3\pi d\eta v \qquad [2]$$

where d is the diameter of the particle, η is the viscosity of air, and v is the velocity of the particle. The velocity at which this resistive force equals the gravitational force is the terminal velocity, v_T.

$$v_T = (\varrho_{part} - \varrho_{air})\frac{gd^2}{18\eta} \qquad [3]$$

For example, for a unit-density, 1-μm particle falling through air at atmospheric pressure and

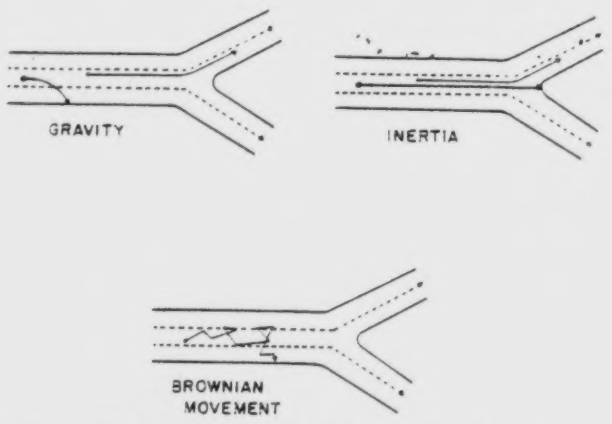


Fig. 2. Schematic representation of aerosol particle motion across streamlines due to the different mechanisms causing deposition. *Upper left.* Settling. *Upper right.* Inertial impaction. *Bottom.* Diffusion.

body temperature, this terminal velocity is 33 μm/s. This terminal velocity would be established in 10 μs if the particle were started from rest.

For unit-density particles settling in air, Stokes' Law is valid (± 10 %) for particles 1 to 40 μm in diameter. Correction factors can be included to extend the useful range for particles 0.001 μm to 200 μm in diameter (129). With large-diameter particles, the retarding force is increased by the inertial effects of accelerating a mass of gas to push it aside. With a small-diameter particle, the retarding force is less than that predicted by Stokes' Law because the particle is comparable in size to the mean free path of air molecules and can slip between molecules. Although the terminal velocity of most irregularly shaped particles cannot be predicted directly from this formula, nonspheric particles are often characterized by an "aerodynamic diameter." This is the diameter of a unit-density sphere having the same settling velocity (v_T) as the particle in question. For such particles, the aerodynamic diameter must be experimentally determined. It is difficult to calculate the effective aerodynamic diameter from the shape and density of the particle.

Inertial Impaction

If a particle moving at velocity, v, is placed into still air in the absence of gravity, the drag forces will bring it to a stop. If the resistance to particle motion is given by Stokes' Law, this "stop distance," χ, can be calculated to be

$$\chi = v\left(\frac{v_t}{g}\right) \qquad [4]$$

where v_T is the settling velocity given in equation 3.

This is analogous to what occurs when an airstream moving at velocity, u, with aerosol particles moving along with it makes a sudden change in direction by an angle, θ. The particle suddenly obtains a velocity of magnitude u sin θ at right angles to the airstream. Hence, by the analysis given previously, the particle moves a total distance of

$$\chi = \frac{(v\sin\theta)\, v_T}{g} \qquad [5]$$

across the direction of flow before it again comes to rest relative to the airstream. If traveling this distance brings it into contact with a wall, the particle is assumed to be deposited.

If a unit-density 1-μm particle travels in an airstream moving at 0.2m/s (typical of centerstream air velocity in bronchi), then a 30° change in the direction of air flow will move the particle 0.3 μm away from its previous streamline. This displacement increases with airstream velocity, angle of airstream deflection, and the square of the particle diameter, which affects v_T.

If we compare χ, the stop distance, to R, the radius of the tube, and replace the peak velocity, u, by twice the average velocity, $\bar{v}$, this ratio is the Stokes' number, which is related to the probability of deposition by impaction:

$$\mathrm{Stk} = \frac{\varrho d^2 \bar{v}}{18\eta R} \qquad [6]$$

Diffusion

The constant random collisions of gas molecules with small aerosol particles pushes them about in an irregular fashion called Brownian movement. Thus, even in the absence of gravity, a particle in still air moves around in a "random walk." This is a probabilistic process that allows motion in 3 dimensions. The root-mean square displacement, Δ, after a time, t, is given by

$$\Delta = \sqrt{6Dt} \qquad [7]$$

where D is the diffusion coefficient of the particle, which can be expressed in terms of T, the absolute temperature; k, the Boltzmann constant; η, gas viscosity; and d, particle diameter:

$$D = \frac{kT}{3\pi\eta d} \qquad [8]$$

A unit-density 1-μm sphere will move 13 μm in 1 s as a result of diffusion. It is important to remember that this displacement varies with the square root of time. As expressed in equations 7 and 8, this displacement increases as particle size decreases, independent of density. A comparison of settling and diffusion for a range of particle sizes is shown in table 3.

Electric Forces

The surfaces of the respiratory tract are uncharged, but electrically conducting. When an electrically charged particle approaches such a surface, it induces an "image charge" of the opposite polarity in the surface and is attracted toward it. This force becomes stronger as the particle approaches the surface, and deposition

TABLE 3

ROOT MEAN SQUARE BROWNIAN DISPLACEMENT IN 1 S COMPARED TO DISTANCE FALLEN IN AIR IN 1 FOR UNIT-DENSITY PARTICLES OF DIFFERENT DIAMETER*

	Diameter (μm)	Brownian Displacement in 1 s (μm)	Distance fallen in 1 s (μm)
Settling greater in 1s	50	1.7	70,000
	20	2.7	11,500
	10	3.8	2,900
	5	5.5	740
	2	8.8	125
	1	13	33
Diffusion greater in 1s	0.5	20	9.5
	0.2	37	2.1
	0.1	64	0.81
	0.05	120	0.35
	0.02	290	0.013
	0.01	570	0.0063

* Temperature = 27° C; gas viscosity = $.19 \times 10^{-4}$ poise. Appropriate correction factors were applied for motion outside the range of validity of Stokes' Law.

of charged particles may be greater than that of neutral ones. If all of the aerosol particles have charges of the same polarity, they will repel each other and again potentially enhance deposition. These forces depend on the amount of charge on the particle; however, this charge is difficult to quantify or to predict. Even when a neutral particle approaches a neutral surface at distances on the order of 1 or 2 μm, electric forces called Van der Waal's forces cause an attraction between the 2. These forces are due to fluctuations in the positions of electric charges in the 2 bodies, and any electric dipole moment formed in one body will induce an attractive dipole moment in the other. These forces, in addition to the fact that most collisions are inelastic, insure that aerosol particles coming very close to or actually contacting a surface will adhere to it. As will be discussed subsequently, it has not been possible to study in a precisely controlled manner the effect of electric forces on deposition in the respiratory tract.

Interception and Other Effects

Even in the absence of any of the mechanisms described, deposition can occur because the inspired air is brought close to a large surface area. As particles move into smaller and smaller air spaces, they may reach a point where the distance to a surface is less than the particle's size; this contact is called "interception." The importance of this mechanism can be estimated by comparing the size of the volume from which deposition by interception can occur to the total lung volume. If the surface area of the lung is assumed to be 70 m^2, then for particles with a radius of 1 μm, collection will occur from the portion of the volume that is 2 μm away from this surface. This is equal to 140 ml, a relatively small fraction of the lung volume. Moreover, this calculation should be corrected for the fact that the undiluted aerosol is not presented to the entire surface of the lung, i.e., it is "filtered" by the bronchi and bronchioles before reaching the major surface area of the lung. In addition, convective flow cannot bring freshly inspired air to within micrometers of respiratory surfaces. However, for particles such as fibers, whose extreme dimensions are large, interception can become an important mechanism.

Other forces acting to effect deposition, such as acoustic, magnetic, thermal, and radiational, are normally not significant in the lung. Two of these, magnetic and acoustic, may be used to enhance deposition experimentally. Enhancement by magnetic forces has been reported experimentally (143). Spontaneously breathing conscious rabbits were exposed to 300 μg of gamma ferric oxide (Fe_2O_3) aerosol/L for 1 h while a 2,000-gauss permanent magnet was applied to the chest. A control rabbit was exposed while a piece of bronze was applied in a similar position to the chest. The magnetic content of the lung was assayed with a magnetometer. An enhancement of deposition as large as 5-fold was obtained in regions near the magnet compared to those farther away.

Characterization of Exposure to Aerosol

Nature of the Problem

To describe completely deposition due to exposure to an aerosol, one would need to list the amount and characteristics of the particles deposited as a function of location in the respiratory tract. As will be discussed later, this is a formidable task; accurate local measurements can be made only by dissecting the respiratory tract into many pieces and then quantifying the amount of material deposited in each. Even this procedure may not be adequate for particles that dissolve rapidly or are moved rapidly from their initial site of deposition by mucociliary transport or cough. One goal of studying aerosols is to be able to predict the distribution of deposition from particle size, breathing pattern, and respiratory system anatomy.

If an instrument other than the lung is used to sample an aerosol, attention must be paid to collecting a representative sample. Flows higher than those found in the lung may break up aggregates or impact particles before they are measured. Flows that are too low may cause large particles to settle out and small ones to diffuse to and collect on the walls. Factors influencing evaporation, condensation, polymerization, conglomeration, agglomeration, and disaggregation must be closely controlled. Also, the collection process should separate size fractions sharply, should not alter the particles physically or chemically, and should collect them in sufficient numbers to allow for physical or chemical analysis.

For a given aerosol, the 2 most important parameters determining deposition are mean diameter and distribution of particle diameters. For spheric particles, the definition of diameter is unambiguous; but for irregular particles, a variety of definitions exist. The most useful definition is the "aerodynamic diameter," even though this measure may bear little relation to the physical appearance of the particle. The aerodynamic diameter is the diameter of a unit-density sphere that has the same settling velocity as the particle in question. The distance that the particle moves in inertial impaction and settling increases with the square of the aerodynamic diameter, whereas motion due to diffusion is inversely related to the square root of the physical diameter. Interception increases linearly with the physical dimensions of the particle.

Description of Aerosol

Complete texts have been written on the characterization of aerosols (129, 133); the following represents a brief summary of the properties of aerosols. Measurement of mass concentration (in mg/m^3) is usually not sufficient to predict deposition. Aerosols are very rarely composed of particles of a single size and shape (monodisperse). More often, the size distribution spans several orders of magnitude with widely varying particle shape. The most complete description would be a tabulation of the number, size, shape, density, chemical composition and physical state, charge, and radioactivity of each particle. Fortunately, such a cumbersome compilation is not necessary for most purposes.

For deposition in the lung, the most relevant characteristic of an aerosol is the frequency distribution of aerodynamic diameters. Depending on which particle characteristic is most important, the aerosol can be described in terms of the distribution of particle number, surface area, or mass as a function of aerodynamic diameter.

The distribution of aerodynamic diameters in an aerosol can be experimentally determined, and a frequency distribution function can be empirically derived. Many heterodisperse aerosols fit a log-normal distribution in which a plot of particle number density (number of particles per given size interval) versus the logarithm of size produces a bell-shaped, normal distribution curve. Such aerosols can be effectively described by 2 numbers, the count median diameter and the geometric standard deviation (GSD). The GSD is the ratio of the diameter of the particle that is larger than 84.2 % of all others to the count median diameter.

In addition to characterization by 2 parameters, a further advantage of the log-normal distribution is the fact that if particle size is log normally distributed, so also are particle mass, particle surface area, and any other properties that are power functions of particle size. Therefore, all have the same GSD. These log-normal distributions are interrelated by the Hatch-Choate equations (134). For example, mass median diameter (MMD) is related to count median diameter (CMD) by

$$\ln (\text{MMD}) = \ln (\text{CMD}) + 3 (\ln \text{GSD})^2 \qquad [9]$$

where ln is the natural logarithm. This shows

that the mass median diameter is larger than the count median diameter, because the larger particles contribute the predominant mass.

The disparity between the various particle parameters disappears with monodisperse particles (GSD = 1) and increases with increasing heterogeneity. The relationships among various particle size parameters when the distribution of particle sizes is plotted on a Cartesian graph are shown in figure 3.

Often, the size distribution of a toxic or therapeutic aerosol is given in terms of "activity." For log normally distributed aerosols, the important parameter in deposition is activity median aerodynamic diameter. The relationship of "activity" to the physical properties of the particle depends on how the pharmacologic or radioactive material is distributed throughout the particle. If it is distributed evenly throughout the particle, then the activity median aerodynamic diameter is the same as the mass median aerodynamic diameter. If it is distributed only on the surface, then the activity median aerodynamic diameter is the surface median aerodynamic diameter. Even if the substance is distributed in some unknown way with regard to particle dimensions, activity median aerodynamic diameter is nonetheless the appropriate parameter in deposition, and the instrument used to determine it must collect fractions of various sizes and analyze them for "activity."

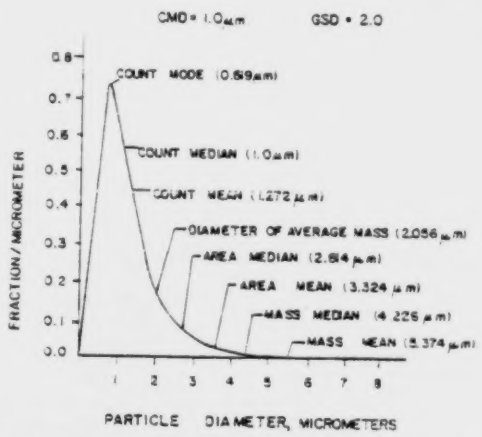


Fig. 3. The size distribution of an aerosol whose particles are log normally distributed. The curve, which is skewed when the diameter of the particle is plotted on a linear scale, is symmetric and bell shaped when diameter is plotted on a logarithmic scale. The various size parameters that can be computed for any log-normal distribution using the Hatch-Choate equations are indicated along the curve. CMD = count median diameter; GSD = geometric standard deviation; μm = micrometer(s).

Experimental Measurement of Aerosol Size Distribution

Respirable mass sampling. Often, rather than collecting the entire aerosol, which may include large, nonrespirable particles, it is useful to use a sampler that mimics the size selectivity of the respiratory tract. The respiratory tract itself could be used as the aerosol sampler, but (*1*) this is not always feasible, and (*2*) a single such measurement does not yield information that can be used to predict changes in deposition as a function of breathing pattern.

A number of mechanical devices do mimic, to some degree, the collection characteristics of the lung or do provide information about the aerodynamic size distribution of the particles. Because coarse particles are predominantly collected in the upper respiratory tract and fine particles are predominantly collected in the pulmonary region, some samplers divide respirable dust into 2 or more fractions, so that deposition in the various regions can be predicted. A discussion of respirable dust sampling has recently been published (135).

Measurement of mass. Sampling of mass concentration (mg/m^3) is easy to do and adequate if particle size is known or if access to particles is restricted to those in the respirable range. Particles can be collected by filtration or impingement on a liquid surface, followed by gravimetric or chemical analysis. Instruments are also available that provide information on concentration of aerosol in the gas phase by light scattering or by β-ray attenuation in a collected sample (GCA Corp., Bedford, Mass.). Aerosols can also be precipitated by a strong electrostatic field, after which mass can be determined. In one instrument (TSI Incorp., St. Paul, Minn.), the aerosol is deposited onto a resonant piezoelectric crystal that changes its resonant frequency in response to the mass collected on it (136).

Measurement of particle size. All size-sensitive sampling relies on some property of the aerosol that changes with size. The most obvious is geometric appearance, measured by light or electron microscopy on representative samples of the aerosol. For example, a point-to-plane electrostatic precipitator can be used to collect

a representative sample of aerosol onto a carbon-coated electron microscope grid. The resolution of the deposited aerosol can be improved by shadowing the grid with chromium or gold in a high-vacuum evaporator. The particles can then be examined in a transmission or scanning electron microscope. From photographs taken of the aerosol, the individual particles are classified, and the characteristics of the resulting distribution are described by standard statistical methods. Often, the diameter of the projected area is measured because it has been related to other properties such as aerodynamic diameter, as shown by Cartwright (137). The diameter of the projected area is the diameter of the circle that has the same area as that of the particle silhouette. Good accuracy and resolution may be obtained by such methods. However, they are extremely time consuming, and if it is essential to predict the aerodynamic behavior of the particles, then their shapes and densities must also be taken into account. Devices that rely on the measurement of light scattered from aerosols can also supply information regarding size distribution. This approach works best when the aerosols are spheric and when the incoming light is homogeneous. Excellent devices using lasers and spectrometers communicating with computers are available (Particle Measurement Systems, Inc., Boulder, Colo.).

The aerodynamic size of particles affects their settling and impaction; both of these processes are used for size-selective sampling. In the Andersen cascade impactor, the aerosol is passed at successively higher velocities through a series of jets directed at collecting discs. Coarse particles are collected at earlier stages; fine particles, either at later stages or in a filter placed at the output of the instrument. Because size selection at each stage is not precise, these devices tend to overestimate the GSD. Samplers that rely on settling are called "elutriators." In these samplers, the particles are introduced in a narrow band of air at the top of a long horizontal channel. Again, coarse particles fall to the bottom early; fine ones, later or not at all.

For submicrometer particles that settle slowly, and for delicate agglomerates that break up easily (and for which the aerodynamic behavior is impossible to predict from electron micrographs because of their complex shape and unknown density), effective aerodynamic diameters can be measured directly using a concentric aerosol centrifuge of the Tillery design (138) with Hinds' modifications (71). This aerosol spectrometer determines aerodynamic size distributions from observations of particle settling velocity in a centrifugal field. This instrument draws a sample of aerosol directly from the exposure area and, as shown in figure 4, enhances particle sedimentation velocity by flowing the sample into a rotating chamber and around a rectangular deposition channel concentric with the axis of rotation. Centrifugal forces arising from high-speed rotation (3,000 rpm with a channel 7.1 cm from the center) cause an acceleration of 700 *g* at right angles to the aerosol flow down the channel. The particles are deposited on a removable strip that lines the outer wall of the channel. This collecting strip can be metal or paper impregnated with a solution of graphite, so that static charges are drained away and will not repel or attract particles as they approach. Aerodynamic size is correlated with the position of deposition along the strip relative to the point where the aerosol entered. Large particles are deposited soon after entry into the channel; small ones, further along the strip. Because aerodynamic size is defined in terms of settling velocity, the point of deposition can be derived mathematically, and the theoretic calculations are found to agree with deposition of polystyrene and polyvinyl toluene spheres of known density and diameter. The strip is cut into segments corresponding to known aerodynamic size ranges and analyzed. Instruments of this kind also exist with the channel in spiral rather than concentric form. The advantage of spiral centrifuges is that a somewhat wider size range can be covered, although the resolution is limited by secondary

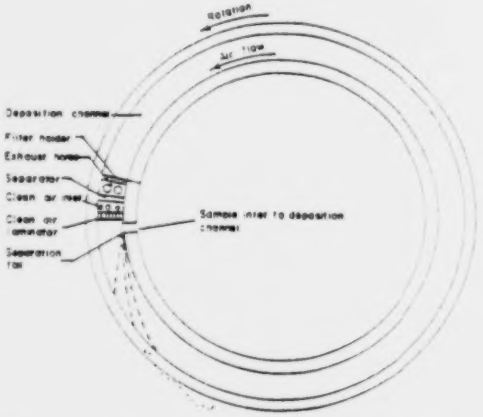


Fig. 4. Schematic view of the rotating head of the aerosol centrifuge.

flows that disturb the settling of the aerosol. The theoretic predictions are also less precise.

The angle and amplitude of light scattering from an airborne aerosol particle varies with particle size and can be used to define an optic diameter. This technique is particularly useful for sizing fluid droplets and in situations requiring instant-by-instant monitoring. The light scattering can be examined for the aerosol in bulk (Tyndall meter) or can be measured for one particle at a time, as in laser scattering systems. One such commercially available aerosol spectrometer (Particle Measuring Systems, Inc., Boulder, Colo.) sizes particles from 0.15 to 3.0 μm in 45 non-overlapping intervals. This size range can be further broken down into 4 smaller but more magnified ranges, each of which is divided into 15 intervals. An on-line computer stores the count-size-interval data, and computes and prints out the resultant frequency-size distribution and the normalized particle size distribution function (probability density). Two disadvantages of this technique are that particles must be sufficiently dilute to avoid oversaturation, and that it is difficult to interpret data from nonspheric particles. In addition, particles less than 0.15 μm in diameter go undetected.

Insoluble particles collected in a fluid can be sized by successive filtrations through Nucleopore filters or by a Coulter counter. The Coulter counter measures the volume of each particle as it passes through a small orifice, thereby restricting the flow of electric current in direct proportion to particle volume.

Other more sophisticated devices are available that charge the aerosol and subsequently measure its mobility in an electric field (TSI Incorp., St. Paul, Minn.). These devices are useful for particles less than 1 μm in diameter. Another device developed by Mazumder and Kirsh (139) measures particle aerodynamic size by determining the response of single aerosol particles to sinusoidal oscillation of the air. This instrument can size 40 particles/s if they are within the range of 0.1 to 10 μm.

For tests of aerosol deposition in animals, design of the exposure chamber is as crucial as characterization of the aerosol. Uniformity of aerosol concentration is essential and must be confirmed with the animals in place. Exposure may be of the whole body of free-ranging caged animals. Alternatively, special restraining devices that expose only the animal's head to the aerosols may be used. As will be discussed later, animal size, age, species, body position, and the level of activity and stress all affect the breathing pattern and thus help determine both the total amount and the site of deposition.

Introducing Particles Into the Lung

Exposure to an Aerosol

Key elements in exposure of laboratory animals to aerosols are generation of the aerosol, characterization of the aerodynamic size and other features of the aerosol, design of the inhalation chamber, delivery of the aerosol, and recognition of the factors that influence the amount of material deposited in the lungs. Different animals breathing the same aerosol may frequently retain quite different numbers of particles.

Principles of Generation

The generation of an aerosol requires considerable input of energy to disrupt cohesive forces between particles and to form a vast new surface area. Coarse aerosols are easier to produce than fine ones. The common processes involved are (*1*) atomization of solutions; (*2*) dispersion of dry powders or pulverization; (*3*) condensation from the gas phase. The energy is usually provided by compressed air or piezoelectric crystals vibrating at ultrasonic frequencies. Often, initial formation is followed by condensation or evaporation to produce particles of the desired size. Alternatively, some size selection can be accomplished by using baffles, holding chambers, or size-selective collection and subsequent re-aerosolization.

The most important generator characteristics are (*1*) median aerodynamic size of the aerosol produced, (*2*) GSD, (*3*) delivery capability, in terms of mass produced per unit time or per unit volume of gas, (*4*) cost, and (*5*) ease of operation. An ideal generator would create aerosols reproducibly, yet would allow the first 3 characteristics to be set within a wide range. Other desirable attributes are reliability and versatility in accommodating a wide range of solvents and solutes.

Polydisperse Aerosols

Some generators atomize a liquid by moving a blast of air or propellant across a narrow nozzle into which liquid has been drawn by the Bernoulli effect. As shown in figure 5, the largest droplets are removed by curving the airstream so that they impact. Removal of droplets larger than 10 μm distinguishes a nebulizer from a

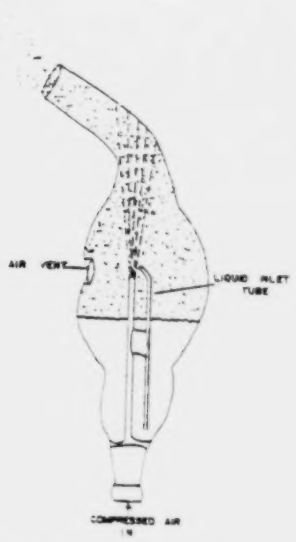


Fig. 5. Schematic of the DeVilbiss no. 40 compressed air nebulizer.

spray atomizer. Well-known nebulizers include the Dauterbande, DeVilbiss, Lauterbach, and Lovelace; all rely on an air jet as the energy source. The size range of primary droplets is from 1 to 10 μm with GSD = 1.8 to 2.0. The outputs are of the order of 1 to 30 μliters of fluid/L of air. Some of the contrasting characteristics of compressed-air nebulizers have been collected by Tillery and co-workers (140).

Ultrasonic nebulizers use a piezoelectric crystal that transforms high-frequency electric oscillations into mechanical oscillations that, in turn, are coupled via a fluid to the liquid to be nebulized (figure 6). The high-frequency turbulence produces a fountain from which aerosol droplets are carried out of the generator by an air stream. The count median aerodynamic diameter (CMAD) of the primary droplets is determined by the frequency of oscillation, and can be predicted by the equation (141)

$$D_{CMD} = 0.34 \left(\frac{8\pi\gamma}{\rho f^2}\right)^{1/3} \qquad [10]$$

where γ is the surface tension of the nebulized liquid, ρ is the density of the liquid, and f is the frequency of oscillation. For example, a 5-MHz oscillation frequency yields particles 1.5 to 2 μm in diameter, depending on the properties of the solution. A comparison of some commercially available ultrasonic aerosol generators has been given by Tillery and associates (140). The output concentration of ultrasonic nebulizers is considerably higher than that of air-jet nebulizers, but this also means that coagulation is more likely.

If the liquid aerosol droplets are allowed to evaporate, then the diameter of the residual particles is determined by the concentration of nonvolatile solids initially present in the solution. The diameter of the particle, D_p, is related to the primary droplet size, D_d, by

$$D_p = D_d \left(\frac{C}{\rho}\right)^{1/3} \qquad [11]$$

where C is the concentration of solute in the solution, in g/cm^3, and ρ is the density of the dried solute. In both compressed-air and ultrasonic nebulizers, the solute concentration may increase significantly with time because of the inevitable evaporation of solvent and reflux of large aerosol droplets that accompanies production of aerosol.

Polydisperse aerosols can also be produced by aerosolization of dry particles. The Wright dust feed (figure 7) is a commonly used device in which a scraper blade sweeps dust from a compacted plug of preformed particles into an air stream. However, it is difficult to redisperse very small particles, and transients in mass concentration and size distribution are possible.

The *de novo* formation of aerosols by such processes as combustion and condensation is responsible for many urban air pollutants. Similar techniques can be used to produce experimental aerosols. Submicrometric polydisperse iron oxide aerosols can be formed by burning iron pentacarbonyl [$Fe(CO)_5$] vapors, as described by Brain and associates (142) and by Valberg and Brain (143).

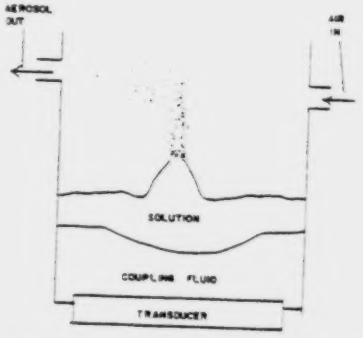


Fig. 6. Schematic of an ultrasonic nebulizer, showing the fountain of liquid from which a fog of aerosol droplets is formed.

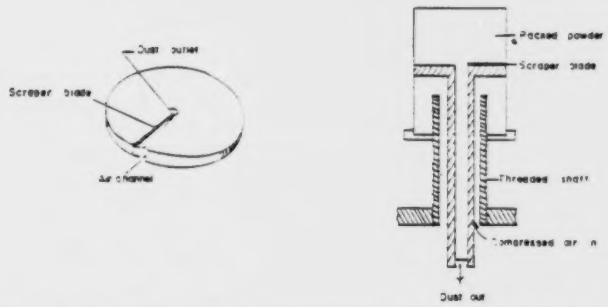


Fig. 7. Cross-sectional view of the Wright dust feed generator and the scraper blade into which the packed powder is fed.

Iron oxide is biologically innocuous and can be easily quantified by standard techniques of chemical analysis, such as atomic absorption, colorimetry, or spectrophotometry. In large amounts, it is visible in unstained tissues as a reddish brown pigment; it can also be detected histochemically by the Prussian blue reaction for iron. Iron oxide particles are efficient absorbers of electrons and appear as dark objects of characteristic appearance in electron micrographs of sectioned cells. A magnetic iron oxide aerosol can also be generated and non-invasively quantified down to microgram quantities by means of a fluxgate magnetometer.

These aerosols are generated by the combustion of $Fe(CO)_5$ vapors in the gas phase. The vapors are brought to the furnace in a gas that has become saturated with the vapors by bubbling through the liquid $Fe(CO_5)$. Iron oxide is produced by mixing $Fe(CO_5)$ vapors with air to provide O_2 and then strongly heating these gases to produce an aggregate aerosol. Iron oxide aerosols of varying size, surface area, and magnetic properties can be generated (143).

Monodisperse Aerosols

Monodisperse aerosols have distributions of aerodynamic diameters with GSD <1.2. The simplest method of producing them is to atomize suspensions of monodisperse particles in pure solvents. Polystyrene latex and polyvinyltoluene spheric particles of specific mean diameter and very narrow size distribution are commercially available. The mean diameters range from 0.09 to 1.17 μm for latex spheres and 1.8 to 3.5 μm for polyvinyltoluene, all with standard deviations ranging from 9 % of diameter for the smaller particles to 1.2 % of diameter for the larger ones. The suspension of particles must be diluted to assure that the proportion of droplets containing more than one particle is small. The degree of dilution necessarily depends on the relationship between primary droplet size of the nebulizer and suspended particle size. In addition, the particle suspensions may contain low concentrations of stabilizers and other impurities. As the droplet evaporates, these impurities will increase the size of the bare particles or form small particles of residue from droplets that contain no spheres. Raabe (144) has discussed the dilution conditions that will produce the greatest number of drops with one particle per drop.

One means of forming monodisperse droplets is the spinning disk aerosol generator. Liquid is fed to the center of a spinning disk that throws a fluid film out to its edge by centrifugal force. When a droplet of sufficient size accumulates at the edge, it overcomes the surface tension forces holding it to the disk and is thrown off. The balance between the force provided by surface tension and the force required to keep the drop in circular motion on the disk is given by

$$\frac{\pi}{6}\,\rho\,D^3\,\omega^2\,\frac{d}{2} = \gamma\pi D \qquad [12]$$

where ρ is the density of the liquid; D, the diameter of the drop; ω, the angular velocity; d, the diameter of the disk; γ the surface tension of the droplet. Hence, the count mean diameter of the droplet (D_{CMD}) is given by (144):

$$D_{CMD} = \frac{2\sqrt{3}}{\omega}\left(\frac{\gamma}{\rho d}\right)^{1/2} \qquad [13]$$

where the definition of ω, γ, ρ, and d are the same as those in equation 12. The actual size of primary droplets may vary by a factor of 2 from this theoretic prediction. In addition, secondary droplets are produced when the primary droplet is

thrown off. These are smaller and less monodisperse than the primary droplets and must be separated from them. Monodisperse aerosols (GSD < 1.2) can be formed by the spinning disk generator in the size range of 1 to 10 μm; however, particle concentrations tend to be low (100 particles/cm³).

Another technique for producing aerosols of excellent monodispersity is the vibrating orifice generator first described by Strom (145) and by Berglund and Liu (146). The ultrasonic vibration of a fluid jet in the axial direction causes it to disintegrate into monodisperse droplets. This generator produces a monodisperse aerosol (GSD < 1.04) in the size range of 0.5 to 30 μm, depending on the orifice used and the conditions of operation. The count median diameter (D_{CMD}) can be determined with an accuracy of better than 2 % from the following relationship:

$$D_{CMD} = \left(1 + \frac{I}{C}\right) \left(\frac{6CQ}{\pi f}\right)^{1/3} \quad [14]$$

where I is the volumetric fraction of nonvolatile impurities in the solution; C, the volumetric fraction of a nonvolatile solute in a volatile solvent; Q, the liquid flow; f, the frequency of oscillation.

Monodisperse aerosols can also be produced by controlled condensation of substances with boiling points within the range 300 to 500° C. A variety of organic substances such as dioctyl phthalate, dibutyl phthalate, esters of sebacic acid, and stearic acid can be vaporized and then collected on small condensation nuclei to form aerosols in the micrometric and submicrometric range. If condensation time is carefully controlled, these can be relatively monodisperse (GSD < 1.2). The Sinclair-LaMer apparatus used for this purpose is shown in figure 8. Particles form in the chimney when the mixture of condensation nuclei and vapor cool. These generators can provide particles in the range 0.05 to 1 μm; as particle size increases, the concentration decreases from 10^8 to 10^4 particles/cm³ (133).

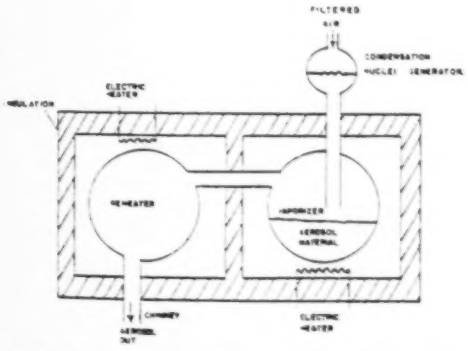


Fig. 8. Schematic of the Sinclair-LaMer aerosol generator used to produce monodisperse aerosols by controlled condensation of substances that can be vaporized in the temperature range 250 to 500° C.

A more complex method of producing monodisperse aerosols is by physical separation of a monodisperse fraction from the output of a polydisperse generator (147). Problems encountered include low mass concentrations and difficulties in resuspending particles collected on a surface.

Aspiration

In addition to being inhaled by spontaneously breathing or mechanically ventilated animals, particles and/or pathogens can enter the respiratory tract by aspiration, which may occur spontaneously or by intratracheal instillation, which can be used experimentally. The dangers of aspiration have long been recognized in surgical and medical emergencies. Drug abuse, shock, drowning, seizures, ethanol intoxication, and general anesthesia are all risk factors (148, 149). Patients with a severe disturbance of consciousness are likely to introduce damaging amounts of material into the respiratory tract by aspiration.

Only recently has it become appreciated that aspiration of oropharyngeal secretions into the lungs is common, but usually well tolerated (150). It is likely that everyone periodically aspirates small numbers of oropharyngeal bacteria that are rapidly cleared by normal defense mechanisms. Persons who snore, the elderly, and those with reflexes depressed by alcohol or drugs may aspirate greater amounts, especially during sleep, although defenses against the aspirate are usually adequate. Interestingly, more than 40 years ago, Amberson (151) noted that contrast material dropped into the mouths of healthy persons during sleep could be detected in the lungs on radiographs the next morning.

In normal persons, the aspirate is composed largely of oropharyngeal secretions, especially saliva, containing bacteria pooled from the tongue, gingival and buccal mucosae, and pharynx. Quantitative cultures of normal saliva yield approximately 10^8 bacteria/ml, with anaerobes outnumbering aerobes by a factor of 3 to

10:1. The number of anaerobes is likely to be even greater in patients with poor oral hygiene; gingivitis exudate, for example, contains concentrations of anaerobes of 10^{11}/ml (150).

The important role of aspiration in infections is supported not only by the production of typical lesions after intratracheal inoculation of anaerobes in experimental animal models (152), but also by the frequent association of anaerobic pleuropulmonary disease with anaerobic dental infection, and the frequent occurrence of these infections in dependent pulmonary segments (153). Furthermore, the microorganisms that predominate in aspiration pneumonia generally reflect the oropharyngeal flora (154).

Intratracheal Instillation

When the method of intratracheal instillation is used, a suspension of particles in a carrier fluid is placed directly into the lumen of the trachea or airway. The fluid and particles, propelled by gravity and sometimes by air flow, run down into the dependent areas of the lung. The carrier liquid is rapidly absorbed into the pulmonary circulation, leaving the particles on the internal surfaces of the lung. Usually, animals must be temporarily immobilized with appropriate anesthesia. Ether or sodium methohexital (Brevital®, Eli Lilly & Co., Indianapolis, Ind.) may be used. The anesthetic effects of this rapidly metabolized barbiturate persist for approximately 5 to 10 min. In large animals such as rabbits, cats, or dogs, a transtracheal injection can be made by locating the trachea a few centimeters below the larynx and passing the needle through the skin, subcutaneous tissue, and the tracheal wall. The correct position of the needle tip is verified by withdrawal of air from the tracheal lumen into the syringe; then the fluid is slowly instilled (155).

In small animals, the needle can be introduced through the mouth. Once anesthetized, rodents such as rats, hamsters, or mice, are placed on a slanted board (20° from the vertical) and supported by an elastic band across the upper incisors. A microscope lamp, with its beam directed at the neck area, provides transillumination (155). By opening the mouth of the animal and depressing the tongue, one can easily see the larynx.

Suspensions of particles can be delivered to the lungs through the trachea with a modified 3-inch spinal needle (bent to form a 135° angle and blunted on the end) inserted between the vocal folds. The presence of the needle in the trachea can be verified at each instillation by gentle movement of the needle within the tracheal lumen to feel the cartilaginous rings. A convenient dose is 0.15 ml of suspension/100 g of body weight. The animals are usually kept upright on the slanted board for 1 min after the instillation (155).

Although delivery of particles by aerosol seems to be more "physiologic," there are some advantages to intratracheal instillation. With intratracheal instillation, the actual dose delivered to each animal can be measured more accurately. In exposure via inhalation, the amount deposited is equal to the product of the minute ventilation and the collection efficiency of the animal. The collection efficiency, in turn, depends on the effective aerodynamic diameter of the aerosol, the breathing pattern of the animal, and the dimensions of the airways and alveoli. With intratracheal instillation, however, the same (measured) amount of material can be introduced into each animal. This procedure is also simpler and minimizes hazards to laboratory personnel when the particles are highly toxic (i.e., carcinogenic or radioactive). For example, experimental lung cancer has been studied in recent years by administering chemical or radioactive carcinogens directly into the trachea. Generation of an aerosol is a complex, sophisticated art involving considerable technical skill; by contrast, intratracheal instillation is much easier to master. The cost of setting up aerosol generation systems and proper exposure chambers may be prohibitive compared to the negligible expense of giving intratracheal instillations. Intratracheal instillation permits the introduction of large and, therefore, effective doses of material within a short period of time. For example, we have exposed rats to 10 mg of Fe_2O_3 by intratracheal instillation. To introduce this same quantity of material by inhalation requires a high concentration of aerosol and a long period of exposure.

Another advantage is that intratracheal instillation eliminates exposure of the skin or pelt. Exposures to aerosols, unless they are exposures of the head only, can result in deposition of large amounts of aerosol on the skin. For some lipid-soluble materials, there may be percutaneous absorption; normal grooming habits may also lead to the ingestion of significant numbers of particles. Highly local exposures are also possible with intratracheal instillation; monolateral or unilobular instillations may be carried out. This is a useful approach when

other parts of the lung are to provide control data to avoid variability between animals.

Intratracheal instillation also allows the introduction of particles that are normally not respirable because of their size. In some instances, it may be difficult to obtain toxic or radioactive metals small enough to be deposited by inhalation in rodent alveoli. With intratracheal instillation, even very large particles (20 to 100 μm) can easily be introduced into the alveolar region. Achieving alveolar deposition in small animals is further complicated by the fact that most are obligatory nose breathers; considerable numbers of inhaled particles may be deposited in the nose and head and rapidly cleared to the gastrointestinal tract. A related advantage of intratracheal instillation is that the site of deposition is relatively independent of particle size. During inhalation, however, inhaled aerosols of different sizes have different sites of deposition.

The primary objection to intratracheal instillation is the high probability that patterns of particle distribution in the lungs may be uneven and unlike those resulting from inhalation. Brain and associates (135) compared the distribution of radioactive particles resulting from inhalation exposures to that resulting from intratracheal instillation; there were important differences. The distribution of particles in the lungs was less uniform after intratracheal instillation. This nonuniformity was partially random, but was also systematic and showed reproducible regional differences caused by gravitational forces. As will be discussed later, some systematic nonuniformities also exist in animals exposed by inhalation of an aerosol; for example, apical regions generally have higher retentions. These differences appear to reflect regional differences in ventilation and collection efficiency. Morphologic studies demonstrated that instillation resulted in heavy, more centralized deposits, whereas the inhalation pattern was lighter and more evenly and widely distributed (figure 9a, b). However, the exact distribution profile varies within certain limits in each case: it is dependent on the volume of the carrier fluid in the case of intratracheal instillation, and it is dependent on ventilatory parameters in the case of inhalation exposure.

Measuring Deposition (Retention)

General Principles

A concept fundamental to respiratory physiology and pulmonary disease is the idea of a dose-response curve. Much attention is devoted to quantifying responses at the organismic, organ, tissue, cell, and subcellular level. Equally important is the careful measurement of dose. In experiments involving natural or experimental exposure to aerosols, measurement of the amount and distribution of retained aerosol is essential.

The distinction between retention (the amount of an aerosol present at any time) and deposition (the initial attachment of suspended particles to a surface) should be kept in mind. Retention, but not deposition, is influenced by clearance and translocation. The exposure to the aerosol must be brief and the interval between death and analysis must be short if the results are to reflect deposition per se. Because of clearance and translocation, there may be loss and redistribution of the deposited particles with time. If the animals are killed, it is important to avoid artifacts caused by agonal and postmortem changes or those caused by fixation and other processes required for the preparation and examination of the specimens.

There are obvious advantages to using nondestructive, non-invasive methods for detecting particle retention. Such approaches allow repeated measurements on the same animal or person, so that replications and serial measurements are possible. For human experiments, such detection methods are essential except when autopsy material is available. However, measurements of retention in whole animals may provide inadequate detail about the distribution of dose to structures of interest. Although gamma cameras, magnetometry, and other non-invasive approaches can give some information of this sort, the greatest precision is obtained by killing and dissecting animals. It is then possible to divide the respiratory tract into individual pieces and analyze the particle content of each piece. Depending on one's patience and the sensitivity of the method of detection, one can describe the distribution of particle retention with varying precision.

Detection of particles can rely on any characteristic property. Particles may be radioactive, fluorescent, radiopaque, or magnetic. They may also have a characteristic visual appearance that can easily be identified in light and electron microscopy. Their chemical nature may be identified by a whole repertoire of chemical techniques, including colorimetry, atomic absorption, neutron activation, and other procedures. The type of question being asked will suggest

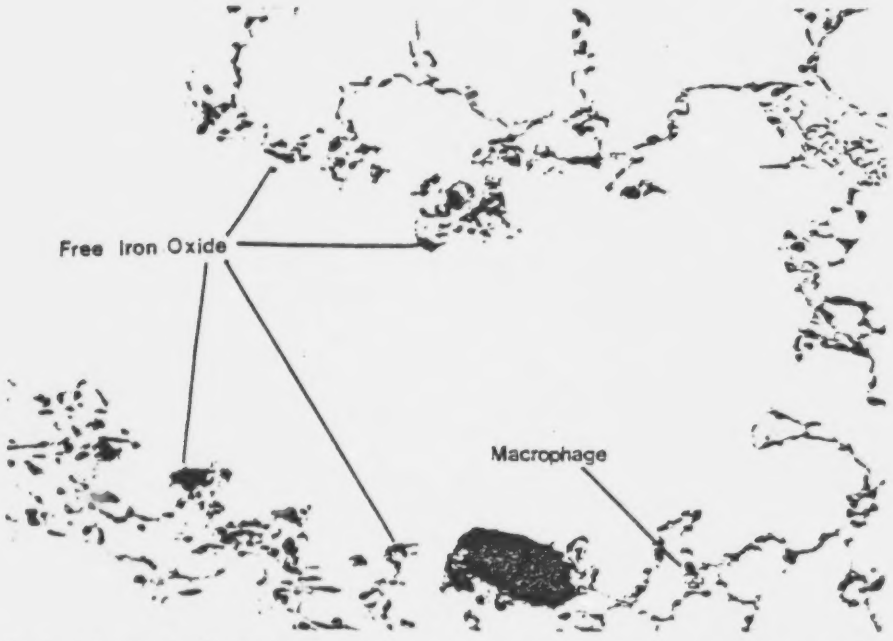
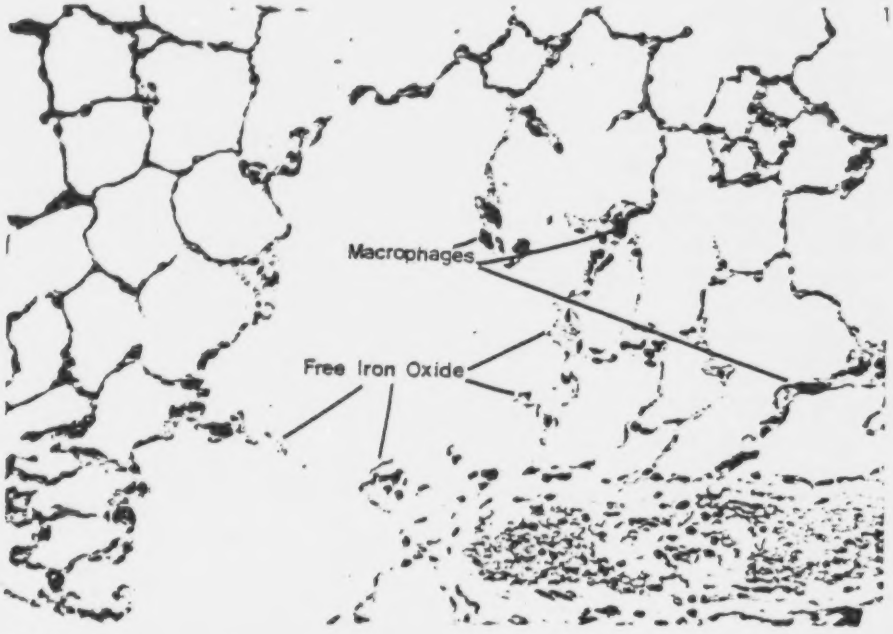


Fig. 9. *A*. Intratracheal instillation of iron oxide particles into hamster lungs 1 h after exposure. Deposition of particles along alveolar ducts is heavy and uneven; few macrophages are present yet (Prussian blue and basic fuchsin, stains; original magnification × 250).



B. Inhalation exposure of hamster lungs 4 h after onset of a 3-h exposure to an aerosol of iron oxide. Deposition of particles is delicate and widespread, although not completely uniform. Many macrophages are engaged in phagocytosis (Prussian blue and basic fuchsin stains; original magnification: × 250).

the properties appropriate for easy, specific detection. Selection of the particle will also be influenced by whether or not external *in vivo* detection of retention is essential.

Estimating Deposition from Exposure

It is possible to estimate dose based on measurements of the size and concentration of the aerosol. In fact, in many published papers, only indices of exposure are provided. Although it is essential to describe the aerosol inhaled, it is important to realize that many factors, such as species, age, activity, and disease, will influence the fraction of the aerosol deposited in each subject.

If ventilation and the size and concentration of the aerosol are measured, it is possible to estimate total and regional deposition, based on theoretic predictions and/or models. Early models for predicting deposition were described by Findeisen (156) and Landahl (157); a more recent one is that of Beeckmans (158). The ICRP Task Group (18) model, based on both theoretic predictions and experimental data, represents the Committee's best estimates of deposition. The model has been converted to convenient equations, nomograms, and computer programs by Brain and Valberg (8), so that total integrated exposure to particles can be calculated when the solubility, size, and concentration of the particle are known.

Inspired versus Expired Concentrations

Another simple approach is to measure the difference in the aerosol content between inspired and expired gas, i.e., its disappearance, and ventilation. Then one can determine the total amount of material deposited in the respiratory tract. However, no information is obtained regarding the site of deposition. Typically, an aerosol photometer is placed in line with the mouthpiece to monitor particle concentration continuously during breathing. The particles in the inspired and expired air pass through an optic chamber illuminated by a light beam. The light scattered by the particles is measured by a photomultiplier tube, and the voltage is amplified and measured. The electric pulse is then proportional to the total light scattered, which, in turn, is a measure of the number of the particles present.

In addition to total deposition in the respiratory tract, some other information can be deduced from such data. If assumptions about the dead space and the extent of mixing are made, the concentration of the particle as a function of expired volume can be used to make some estimates of regional deposition (159, 160); however, such estimates are difficult to validate.

The particle concentration profiles in exhaled air after a single-breath inhalation (with and without breath holding) have also been used to study gas transport within the respiratory tract (86–90). All of these investigators (86–90) used submicrometric aerosols and single-breath inhalations to test ideas concerning mixing processes in the lungs. Submicrometric particles were selected because of their minimal intrinsic mobility due to settling and diffusion; the particles serve, to some extent, as a tracer for gas flow patterns within the lung. Palmes and co-workers (90) have also used persistence of aerosols during breath holding as a way of estimating alveolar dimensions.

Radioactive Tracer Techniques

Radioactivity has been used frequently for studies of aerosol deposition because of its potential for non-invasive measurement as well as its sensitivity and resolution. Hundreds of radioisotopes are produced, and labeled compounds of every sort can be synthesized with a wide spectrum of energy, type of radiation (alpha, beta, and gamma radiation, positrons, and neutrons), half-lives, and doses. Several recent books summarize the applications of radioactivity to imaging (161, 162).

The experiment being carried out and the type of detection system to be used dictate the choice of isotope. Gamma emitters are used most frequently because of their ability to penetrate tissue and their suitability for external measurements. Isotopes emitting gamma rays intended for external imaging should have an energy of at least 20 keV. If they have less energy than that, the major portion of the energy will be absorbed within the body. On the other hand, if the energy exceeds approximately 600 keV, then effective collimation will be difficult, and resolution will be poor. For most gamma cameras and scanning devices, the ideal nuclide should have an energy between 20 and 200 keV. With high energies, problems of shielding and collimator design increase. Alpha or beta emitters are more appropriate for autoradiographs: their short path length, which makes them unsuitable for external detection, produces high resolution on film in contact with particle-laden tissue. Besides method of detection, half-life (and the associated dose to the

animal or patient), cost, and availability should also be considered when choosing an isotope.

Crystals, collimators, and scanners. Radioactive particles or rays can be measured because they interact with matter and transfer energy to a detector. Certain materials emit a weak flash of visible light after absorbing energy from ionizing radiation. The intensity of the light emitted is usually related to the energy absorbed. It is important that the light created within the detector be transmitted and reach the sensitive light detector or photomultiplier tube. It is also important for the intensity of the light flash to die very quickly so that additional events can be detected. Sodium iodide crystals (NaI) are ideal with respect to most of these criteria and are commonly used.

Frequently, it is useful to restrict the field of view of the detector so that only the region of interest is seen. This is achieved by using a collimator constructed of lead that blocks most radiation, but allows radiation from one region of the body to reach the detector. Shields range from a simple collimator consisting of a single cylindric hole in a lead block to elaborate multihole collimators. The size of the holes is critical, and resolution becomes increasingly better as the holes become smaller. However, for a fixed crystal size and shield thickness, as the holes become smaller, the sensitivity of the detector is decreased. A collimator is similar to the lens of a camera; it plays an important role in determining sensitivity, spatial resolution, and depth of field.

A variety of shielded whole-body counters use large NaI crystals and appropriate lead shielding. It is not necessary to surround the subject completely. In some cases, the animal or human subject can be placed on a platform that is slowly moved along under the detector at a constant rate. This provides some information regarding the distribution of radioactivity in the body. One can distinguish material deposited in the upper airways, lung, and gastrointestinal tract (163). Many devices use multiple detectors. Morsy and co-workers (164) have described a system of 4 cylindric NaI crystals that provide excellent sensitivity and particle discrimination. In a rectilinear scanner, a collimated detector is swept in straight lines over the animal or patient; the recording of the count rate is synchronized to the position of the detector. Such scanners have largely been rejected because of the time required to carry out a study.

Gamma cameras. In general, it is more practical to use a stationary detector that simultaneously collects information from a large area. There are numerous versions of gamma cameras, but the most commonly used is that initially developed by Anger in 1956 (165). This device uses a single, large (approximately 25-cm diameter) scintillation crystal masked with a honeycomb, parallel-hole lead collimator containing as many as 15,000 holes. Each part of the crystal looks at a small area of the patient or animal, and scintillations are produced in different parts of the crystal. An array of approximately 3 dozen phototubes 2 inches in diameter senses the light pulses produced in the crystal by incoming gamma rays. The relative pulse sizes from different photomultiplier tubes give the position of the scintillation. From this information, the position of the particle in the respiratory tract can be estimated and then displayed on a cathode ray tube screen or stored in a computer. The distribution of activity in lungs or lung slices can be determined from gamma camera pictures taken using a high-resolution collimator interfaced with a computer. The samples are counted until a total of approximately 500,000 counts is accumulated; the result is a radioactivity "picture" on a color cathode ray tube (figure 10).

The picture consists of an array of 128 by 128 elements; each element represents an area approximately 3 by 3 mm. The counts sensed in each of these elements are recorded on a magnetic disk file for later retrieval and examination. The counts also are converted to color on an intensity scale ranging from blue (for cold) to white (for hot), and the appropriate color is displayed in the radioactivity "picture." Many computer systems allow identification of "regions of interest" on each picture by means of a joystick-controlled cursor. The activity in regions in such a way can be correlated with activity in various anatomic compartments.

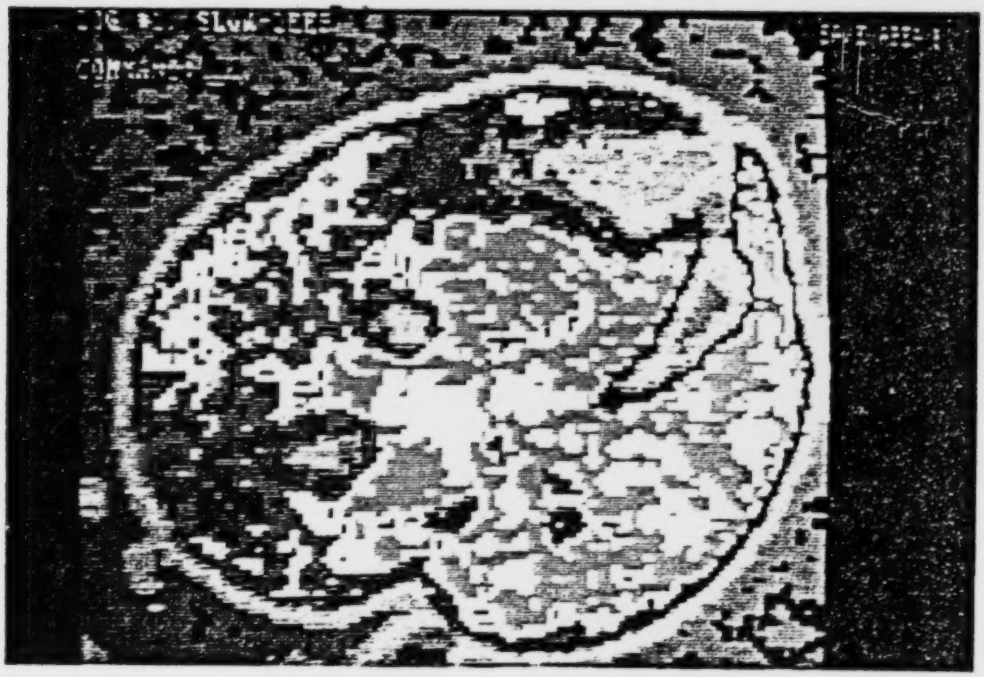
Most systems can detect and separate the simultaneous presence of 2 isotopes by differentiating their gamma ray energy spectra. Once an activity picture is taken with each isotope, the computer system permits addition, subtraction, multiplication, and division of these images so that differences in deposition pattern between the 2 isotopes can be detected. The resolution of the camera depends on the gamma ray energy, the collimator design, and the distance from the collimator to the source. The smaller the distance, the better the resolution.

The choice of radionuclide depends on the type of detector system being used and how important it is to minimize the dose. Isotopes with long half-lives are useful for following particle clearance, but doses to the animal or human subject are larger. Gamma cameras have much lower efficiencies than other detector systems; the efficiency of counting is decreased to approximately 5,000-fold less than that for a typical gamma-well counter.

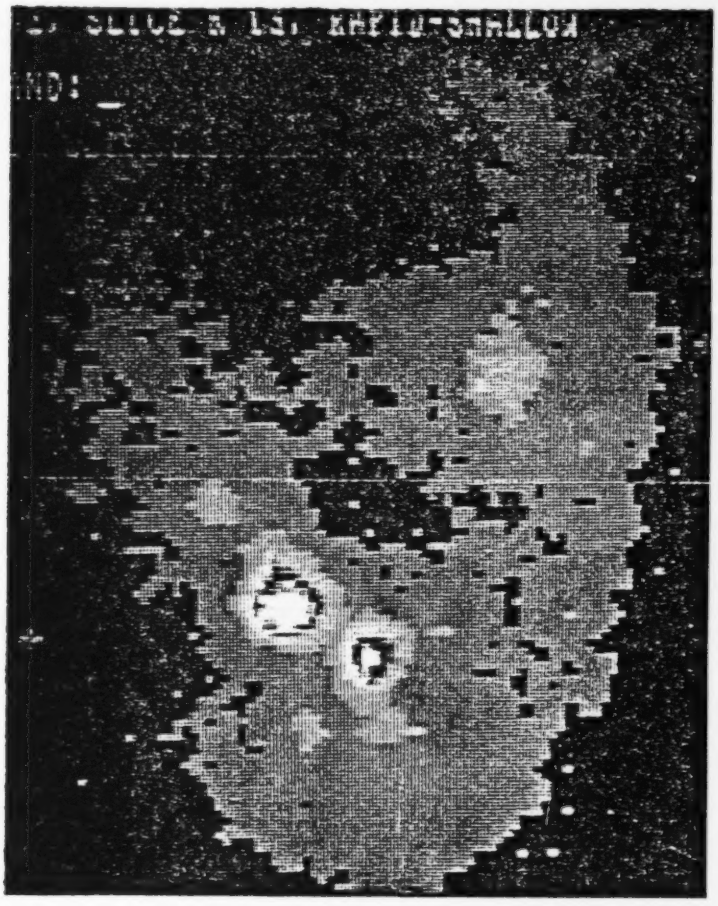
Autoradiography. If slices of lung are placed in contact with film, radioactive particles retained in alveoli and airways will expose the film. A book by Herz (166) provides a thorough discussion of the effect of ionizing radiation on photographic emulsions, and Rogers (167) has recently reviewed autoradiographic techniques. Alpha-emitters or low-energy beta-emitters, whose radiations have a short range in tissue and film, provide the best resolution. In addition, the thinner the specimen and the thinner the photographic layer, the better the resolution.

Resolution also depends on the properties of the film and the geometric arrangement of the film and the source of radiation. The smaller the distance between the source of radiation and the film, the better the resolution. Cember (168) used autoradiographs to examine the distribution of radioactive cesium-144 in the lungs of rodents after intratracheal instillation. We (169) have also used autoradiographs to describe patterns of aerosol retention using ^{99m}Tc. Although it is a gamma emitter with a low-energy (140-keV) gamma ray, ^{99m}Tc also emits low-energy electrons (1.6, 1.9, and 119 keV). Because these electrons are strongly ionizing, they are the predominant source of film exposure. Typically, 10^9 gamma rays are required/cm^2 of film to achieve unit optical density, whereas only 10^7 electrons/cm^2 are required to produce the same density. The short range of the electrons (90 μm in film) results in a crisp image of the distribution of radioactivity over the face of the slice (figure 11). The portions of the lung slice beyond the range of the electrons contribute negligibly to the exposure.

The developed film is a compact record of the site(s) of deposition, with anatomic features readily visible. With an overlay tracing of the lung slice, the distribution of the retained dose can be mapped precisely. To convert this record into meaningful quantitative information, a computer-assisted correlation of anatomic fea-



10A



B

Fig. 10. Photograph of the cathode ray tube output of the gamma camera, showing the distribution of radioactivity over the face of 2 slices of dog lungs. Each picture is a matrix of 128 by 128 cells that shows the activity with white as hot and blue as cold. *A*. Uniform deposition in parenchyma, with cold airways. *B*. Hot airways and cold parenchyma.

tures with film density can be achieved using a laser scanning microdensitometer.

At least 3 problems with autoradiographs require attention. (*1*) During autoradiography, formation of an image from nonradioactive sources, such as fogging, mechanical friction, static electricity, and chemicals in the lung slice, must be avoided. (*2*) To quantify density, the relationship between the optical density of the film and the photometer reading must be determined, and optical density and radiation exposure must be calibrated. (*3*) The anatomic areas must be matched with the same regions in the density record. We (132) have solved this problem by using plastic overlays placed on the autoradiographs. Metal bolts can be used to ensure maintenance of the proper position. By comparing photographs of the slice with the autoradiographs, areas of interest can be outlined on the overlay and identified for the computer-controlled microdensitometer using a sonic pen.

Other approaches using radiation. Radiation can also be used to examine retention of nonradioactive particles. Neutron activation can be used to make a variety of elements radioactive. In addition, antimony oxide absorbs x-rays; this property has been used to estimate antimony trioxide dust retention in the lungs of workers (170). Tantalum (94) and iron ox-

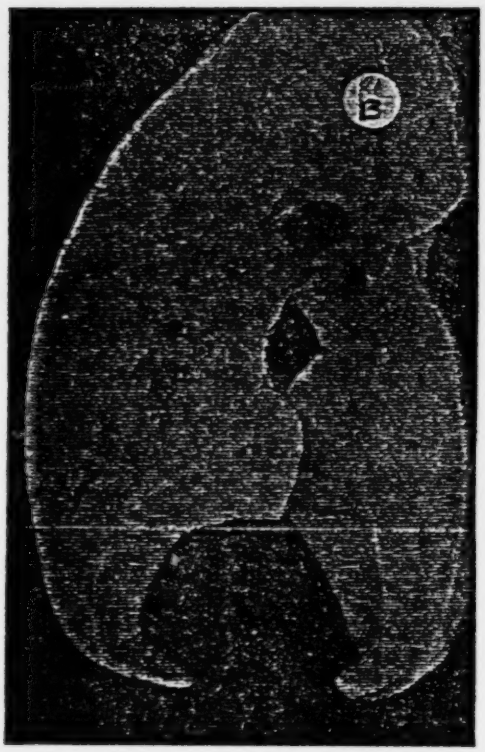


Fig. 11. *A*. A slice of dog lung exposed to a ^{99m}Tc-labeled aerosol and dried.

Fig. 11. *B*. An autoradiograph of the slice of dog lung shown in figure 11A; obtained by putting the slice of lung in contact with X-ray film for 10 h and developing the image produced by the emitted electrons. Dark areas represent high levels of activity (deposition); white areas, low levels of activity. Airway deposition predominates in this slice.

ide (143) also attenuate x-rays and have been used to visualize deposition of aerosol in airways. Friedman and co-workers (171) have used bismuth trioxide to mark teflon discs and have located them with roentgenographic methods.

Magnetic Methods

Magnetic dusts detected with sensitive magnetometry can be used to quantify retention of particles non-invasively in the lungs of animals and man (172–176). Serial measurements of the amount of dust remaining in the lung can be used to describe clearance curves. The greatest advantages of this technique are that it is safe, and that measurements can be made as long as sufficient dust remains in the lung. Thus, one can non-invasively describe clearance kinetics over years. This would be difficult to do otherwise, unless long-lived radioactive materials were used; then, one would face numerous hazards.

Two magnetic dusts are suitable for studying retention of dust in the lung: Fe_3O_4 (magnetite) and gamma-Fe_2O_3 (a magnetically active form of hematite). Both are harmless in small amounts and relatively insoluble at physiologic pH. These materials produce a new magnetic field in response to the application of an external magnetic field. More important, they are ferromagnetic; that is, they maintain part of their new field after the applied field has been removed; this maintained field is called the remanent field. The magnitude of the remanent field can be used to quantify the amount of dust remaining in the lungs.

The sizes of the magnetic particle that we have used are on the order of a few micrometers and are therefore multidomain: each particle behaves macroscopically like a small bar magnet; each particle can be magnetized in any direction with respect to its boundaries, or be

demagnetized. To describe the curve of clearance of dust from the lung, measurements are begun by magnetizing the particles *in vivo* to produce a remanent field external to the body. Either a small cobalt-samarium hand-held magnet or an electromagnet consisting of 2 large copper coils powered by a DC power supply can be used. The saturation level of magnetic dust in tissue is approximately 900 gauss. At lower levels, the strength of the remanent field is related to that of the applied field; but at higher levels, the remanent field no longer increases.

When tiny amounts (0.02 to 0.1 mg) of dust are present, a sensitive magnetic detector called the Superconducting Quantum Interference Device (SQUID) can be used (172). When larger amounts (> 0.1 mg) of dust are present in the lungs, a more convenient (and less expensive) fluxgate detector is appropriate (174). The fluxgate magnetometer has at least one probe approximately the size of a cigarette that measures the component of the magnetic field along its axis. Most units have 2 identical probes, and the output is the sum of both probes. When the probes are placed parallel, but in opposite directions, the instrument functions as a gradiometer, measuring the difference in one component between the 2 probe locations. This allows distant background disturbances to be largely canceled out while the nearby source to be measured produces a much stronger field in one probe than the other. The gradiometer configuration decreases background noise sufficiently so that simple or no shielding suffices. The remanent magnetic field is then used to estimate the amount of magnetic dust present in the lungs.

The deposition of 1 to 3 mg of Fe_3O_4 is sufficient to describe a clearance curve in rabbits with the fluxgate magnetometers (143) or in humans with the SQUID (176). Clearance curves for human smokers and nonsmokers are shown in figure 12. The level of the magnetic field measured with the SQUID is on the vertical axis, and the time elapsed after exposure to Fe_3O_4 is shown on the horizontal axis.

To determine each point on the clearance curve, the particles in the lungs were first magnetized with an external magnetic field. After the external field was removed, the remanent field was measured by the SQUID detector in a magnetically shielded room at the Massachusetts Institute of Technology (176). After magnetization, the magnetic field over the chest produced by the particles decreased continuously, to approximately 15 % within 1 h. This was due to random, small rotations experienced by the particles in the lungs that decreased the vector sum of the magnetic fields of the individual particles. The character of the relaxation curve may provide information regarding the intrapulmonary location of the particles. The relaxation curve is reproducible, with its shape and asymptote varying as a function of time after exposure to the particle. Whenever the amount of dust in the lung was to be measured, we made the field measurements soon after magnetization and for maximal accuracy, extrapolated the relaxation curve back to zero time.

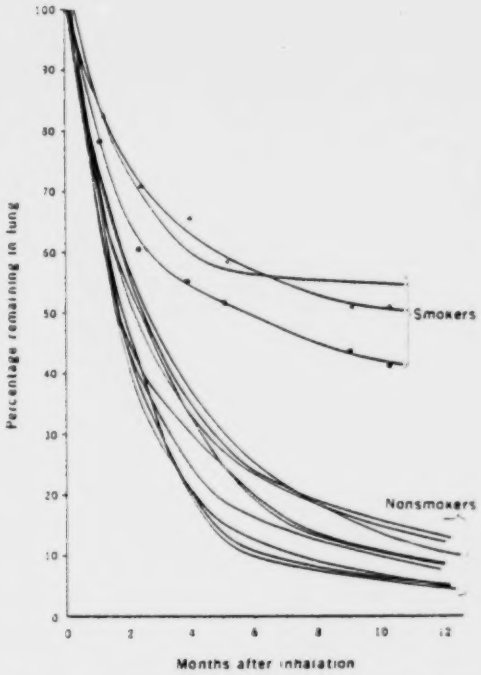


Fig. 12. Long-term clearance curves for magnetic dusts. After 11 months, the smokers retained in their lungs approximately 5 times more Fe_3O_4 dust than the nonsmokers. Experimental points are shown on 2 curves only, to indicate typical scatter. [From Cohen and co-workers (176); reprinted by permission of *Science*. Copyright 1979 American Association for the Advancement of Science.]

As figure 12 demonstrates, magnetic dusts can be used to describe the long-term clearance of dust from human lungs for a year or more. Interestingly, this suggests that clearance of dust from the lungs of cigarette smokers is considerably slower than that of nonsmokers. After approximately 1 yr, 30 % of the dust originally deposited remained in the lungs of the smokers, whereas only 10 % remained in the lungs of the

nonsmokers. The smokers, therefore, retained 5 times as much dust as nonsmokers.

This method appears to hold great promise, although some details need to be clarified. Because readings are very sensitive to distance, it is possible that redistribution of particles within the lung after exposure might alter the measured external field. It is also possible that some of the iron initially deposited may still be present in the lung, but has been converted to nonmagnetic forms. Some work in our laboratory (177) has shown that the amount of ferritin and hemosiderin in the lungs of mice increases after exposure to a submicrometric aerosol of iron oxide. Additional investigations are continuing.

Dissection

Conventional approaches for describing deposition of aerosol in the lung lack adequate resolution to answer many important questions about the spatial distribution of deposited particles. When used with whole lungs or intact animals, collimated NaI crystals and gamma cameras can provide some information regarding the amount and distribution of inhaled deposited aerosols. In a gamma camera picture of a dog or human exposed to a radioactive aerosol, one can easily identify the lung contours, the dome-shaped diaphragm, and the space occupied by the heart. Large shifts in the distribution of the aerosol can be detected, but many important questions cannot be answered by examination of the whole lung. For example, it is nearly impossible to subdivide deposition into airway and parenchymal compartments or to describe gradients from apex to base. Interlobar differences cannot be detected, and it is impossible to identify nonventilated areas when they are small.

We believe that far greater precision and resolution are available when lungs are made rigid by drying or freezing and then sliced or dissected. More important, these approaches permit correlating the site of retention with the anatomic location. Usually, the lungs are removed from the animals, weighed, cannulated, and allowed to dry overnight while inflated with air at a transpulmonary pressure of 30 cm H_2O. Small pieces of waxed paper inserted between the lobes during the drying process prevent sticking, so that the individual dried lobes can be separated easily. Drying time can be decreased to 30 to 60 min if the inflated lungs are irradiated inside a microwave oven. This technique is valuable when short-lived isotopes such as ^{99m}Tc are used. When we were concerned about the precise position of the lungs *in situ* in a given body position, we froze each whole animal overnight, with the lungs inflated to a known transpulmonary pressure, until the lungs and carcass were rigid.

Several approaches for slicing the rigid lungs can be used. In each instance, it is important to dissect the lungs in a predetermined and reproducible fashion. The position of the lungs in the intact animal can be estimated by radiographs obtained with the animal in positions characteristic of the exposure period. Whole lungs of animals can be sliced into sections 1 cm thick using either a meat saw or "bologna slicer." A bread slicer, which cuts the entire lung simultaneously and accurately preserves the relative orientation of each slice. Inflated lungs are sometimes embedded in polyurethane foam, which decreases the possibility of personal injury and also permits precise orientation of the lungs during slicing. These slices are further subdivided into pieces as needed or are used with the gamma cameras or autoradiographs. Alternatively, the lobes can be divided and sliced in a plane approximately perpendicular to the axis of each entering lobar bronchus. Each slice can then be subdivided into additional pieces. A final approach is parenchymal stripping. Bits of parenchyma can be picked away from the airway tree to expose it. The airways can then be dissected, and the precise position of individual samples can be recorded. This strategy is appropriate for describing patterns of particle deposition along airways.

When analyzing the aerosol content of pieces of lung, we frequently want to determine whether the particles are evenly distributed throughout a slice or lobe of lung. We have approached this problem by calculating an evenness index (155). The evenness index is calculated by taking the corrected number of cpm/mg of dried or frozen lung for each piece and comparing this number to the activity of the whole lung. The formula for the evenness index (EI) of pieces of lung is:

$$EI = \frac{(\text{cpm/g of lung piece}) \times 100}{\text{cpm/g of whole lung}}$$

An evenness index of less than 100 % indicates that the piece of lung in question received less than an average share of radioactivity; similarly, a piece with an evenness index greater than 100 % received more than an average share of radioactivity. The degree of departure from

100 % indicates how unevenly the particles are distributed. The evenness index can also be calculated for the slices and lobes of each lung. It can be useful to consider the fraction of each piece that is composed of airways. When we considered airway score in pieces of dog lungs, we could account for some of the variability in the data and obtained useful insights into deposition distribution.

Morphologic Methods

Even with analytic approaches that depend on separating the lung into pieces, some questions still cannot be answered. It may be important to know with a precision of a few millimeters where the particles are in the lungs. For example, are particles still free or have they been ingested by pulmonary macrophages? It may be essential to locate an aerosol within a particular cellular organelle. Then, morphologic studies using light and electron microscopy can be very useful. Figures 9A and 9B compare the distribution of iron oxide particles given by inhalation and intratracheal instillation. This kind of information is not easily obtained by other than morphologic methods. There is increasing evidence (177, 178) that inhaled aerosols may penetrate airway and alveolar barriers. Precise location of particles within the bronchial epithelium and beneath it in lymph nodes, and the relationship between these particles and components of the immune system can only be demonstrated with morphologic techniques. Thus, it is important to vary the approach used according to the type of information required.

Factors Influencing Deposition of Aerosol

As described in the foregoing sections, it is possible to identify the physical mechanisms that cause deposition of particles from inspired air. Techniques are also available to measure the size distribution of the aerosol and other characteristics of the particles. But, by far, the most important information we need is a measure of the deposited dose and its distribution in the respiratory tract. As yet, no comprehensive theory exists for predicting dose, because of the constellation of variables that affect the results. Size, shape, and density of the particles; respiratory anatomy; breathing pattern; dead space volume; vital capacity, and disease state are some of the determinants of deposited dose. Moreover, the distribution of the deposited dose also depends on such factors. On the one hand, even though the respiratory system traps nearly 100 % of particles more than 5 μm in diameter, few of these penetrate to the alveoli. On the other hand, the efficiency of alveolar deposition is large if the particles are submicrometric and if deposition in the airways is minimal. Hence, describing the deposition of a heterodisperse aerosol can be complicated, and many deposition studies have lost their value because one or several of the parameters just described were not specified.

Theoretical Results

The anatomy of the respiratory tract determines the proximity of surfaces, angles of airflow deflection, and, to some extent, the amount of time an aerosol particle remains in the system. These factors, in combination with the deposition mechanisms discussed earlier, contribute to empiric equations describing collection of particles in the respiratory tract.

In airways, at an angle to the horizontal, θ, deposition due to settling is controlled by the settling velocity, v_T (equation 3), the time spent in such an airway, t, and the radius of such airways, R. Landahl (157) worked out the following expression for the probability of deposition due to settling (P_S):

$$P_s = 1 - e^{-0.8v_T t\cos\theta/R} \qquad [15]$$

Because the time spent in the airway is inversely related to the through-flow velocity, deposition by means of sedimentation can be viewed as controlled by the ratio of settling velocity to through-flow velocity.

Inertial impaction is governed by the Stokes' number, St, which is the ratio of the stop distance, χ (equation 4), of a particle moving relative to the air stream, to a characteristic dimension of the system, R.

$$St = \frac{\chi}{R} = \frac{uv_T \sin\theta}{gR} \qquad [16]$$

where u is the airstream velocity, v_T is the settling velocity of the particle, θ is the angle of change in direction of the air stream, and g is gravitational force. Landahl (157) has proposed that the probability of deposition of a particle (P_I) due to impaction is given by:

$$P_I = \frac{St}{St + 1} \qquad [17]$$

where St is the Stokes' number.

The deposition due to diffusion, like sedimen-

tation, is influenced by the amount of time spent in the respiratory system. This is shown in the expression for displacement, Δ (equation 7), due to Brownian motion. Again, the characteristic dimension of the air space is key. Landahl (179) has developed a relationship for the probability of deposition due to diffusion (P_D):

$$P_D = 1 - e^{-0.58 \frac{\Delta}{R}} \quad [18]$$

where R is the radius of the airway.

The usefulness of equations 15 through 18 lies more in illustrating how particle size, lung anatomy, and air flow interact to affect the probability of deposition than in precisely predicting respiratory deposition. Wide variations in intralung geometry, unpredictable variation in air flow as a function of breathing cycle and position in the lung, and nonuniform ventilation and distribution of the aerosol are some of the complicating factors that prevent predicting deposition from these principles.

For the purposes of describing deposition of an aerosol, it is customary to divide the lung into 3 anatomically distinct compartments: nasopharyngeal, tracheobronchial, and pulmonary. The nasopharyngeal compartment extends from the nostrils to the larynx. The nasal passages are complex and have narrow cross sections. Hence, factors such as airstream velocity and directional changes are large here, and inertial impaction is correspondingly large. Moreover, in humans, nasal flow is always turbulent (as opposed to laminar); this also enhances inertial deposition.

The tracheobronchial compartment consists of the trachea (approximately 2.2 cm in diameter) down through terminal bronchioles (0.7 mm in diameter). It is an asymmetric system of dichotomously branching tubes, the anatomy of which has been well described by Horsfield and co-workers (180). Inertial impaction is an important mechanism of deposition at bifurcations, and settling can be important in this compartment because of the increased proximity of surfaces. As in the nasopharyngeal compartment, diffusion is less important because of the short residence times of the particles.

The pulmonary compartment includes the respiratory bronchioles, alveolar ducts, and alveoli. The total surface area is immense (70 m^2), and airspace dimensions are small (200 μm). Because bulk movement of gas is very slow, inertial impaction is ineffective as a mechanism for deposition. Sedimentation can occur and, of course, diffusion is an effective mechanism for small particles.

Size Distribution of Aerosol

The most important compendium of the influence of aerosol size distribution on lung deposition has been that of the ICRP, published in 1966 (12). The Committee's conclusions incorporated the models originally developed by Findeisen (156) and Landahl (179). The Committee surveyed a large number of studies of deposition, both experimental and theoretical. A graphic summary of their findings is shown in figure 13. Again, the 1-μm unit-density particle is a convenient reference point. Forty per cent of particles this size are removed by the respiratory system at resting breathing rates. For larger particles, deposition increases, reaching nearly 100 % for 10-μm particles. For particles smaller than 1 μm, deposition remains constant within a small range, with a slight minimum for 0.5 μm particles. It then increases again for particles less than 0.1 μm in diameter. The distribution of this deposited dose in the respiratory tract also varies with particle size (figure 13). One half of the total deposition of the 1-μm particles is deposited in the pulmonary region. Particles larger than 1 μm tend to be deposited before reaching the pulmonary compartment; particles less than 1 μm are deposited less avidly, except for those smaller than approximately 0.1 μm, for which pulmonary deposition increases steeply. In fact, experiments show that for these small aerosols, alveolar deposition is greater than 90 %, and most of the exhaled particles come from dead-space air (181). The range of these curves has been extended by Morrow (182) to include particles of aerodynamic diameter 0.001 to 1,000 μm.

The explanation of these predictions is that, for large particles, inertial impaction and settling are the major mechanisms of deposition; these influences tend to remove particles from inhaled air while it is still in the nasopharynx and airways. For very small ($\leq$ 0.1 μm) particles, diffusion becomes important and deposition increases steeply, particularly in the pulmonary compartment, where the distances to a surface are short and residence times are long.

It should be kept in mind, of course, that the deposition curves are rough estimates, and more precise results require specification of additional variables. In general, large differences in deposition of the same aerosol can be expected among

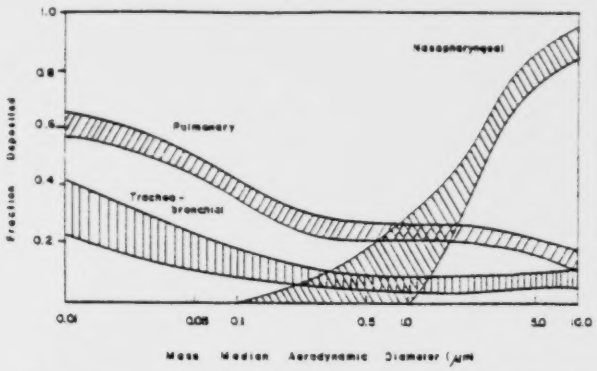


Fig. 13. Deposition in the respiratory tract, as predicted by the International Committee on Radiation Protection model for 3 compartments, shown as a function of the mass median aerodynamic diameter (MMAD) of the aerosol plotted on a logarithmic scale. The amount by which widely different geometric standard deviations affect the curves at a given MMAD is depicted by the width of the curves. [From Morrow (12); reprinted by permission of publisher.]

subjects. However, the ICRP group did conclude that deposition was most closely linked to median aerodynamic size, and that heterodispersity, as measured by GSD, did not affect the results very much.

Lippmann (10) has collected data from his own investigations (183) and those of others on total respiratory tract deposition in humans, based on the difference between inhaled and exhaled concentrations. Despite a fair degree of scatter, the pattern that emerged was a minimal deposition of 20 % for particles in the range 0.5 to 0.7 μm in diameter, with an increase to 100 % for 10-μm particles and an increase to 30 % for 0.1-μm particles. The data showed generally lower deposition than that predicted by the ICRP model. Lippmann and Albert (183) also separated tracheobronchial deposition from pulmonary deposition by assuming that the tracheobronchial compartment is represented by the fraction of aerosol that clears in 24 h. Tracheobronchial deposition decreased monotonically as particle aerodynamic diameter decreased from 10 to 2 μm. Deposition in alveoli was nearly constant at 20 % for 0.1- to 4-μm particles during nose breathing; but during mouth breathing, it reached a peak of nearly 50 % for 3-μm particles. As will be discussed in a subsequent section, these results were influenced to some extent by breathing pattern. Palmes and Lippmann (13) and Giacomelli-Maltoni and coworkers (184) have also pointed out that intersubject variability can decrease the accuracy of predictive models to no better than ± 40 %. Foord and associates (185) have investigated deposition of 2.5- to 7.5-μm monodisperse particles in humans. For this size range, the total percentage deposition increased from 70 to 90 %, whereas the ratio of tracheobronchial to pulmonary deposition, as assessed by 24-h clearance, increased from 0.2 to 2.3. Davies and co-workers (186) have developed a formula that expresses total deposition of 0.5- to 2.5-μm particles as a function of tidal volume, breathing frequency, particle size, and one parameter that is subject specific. Within this size range, deposition increases from 10 to 60 % with increasing tidal volume and decreasing breathing frequency. This type of closely controlled study is important in pinpointing sources of intrasubject variation in deposition. Heyder and co-workers (187) studied total deposition of aerosol for the size range 0.2 to 1 μm while respiratory pattern was closely controlled and no respiratory pauses occurred. Among 4 subjects, they found very little intrasubject variation and a deposition fraction of 0.1, independent of particle size.

Quantitative information on the distribution of deposition within the lung is important both for therapeutic and pathogenic aerosols. Schlesinger and Lippmann (188) compared particle deposition patterns in a hollow cast of the human tracheobronchial tree to published reports on the sites of origin of primary bronchogenic tumors. They noted a close correspondence between relative deposition efficiency in the cast and frequency of reported cancer. Data on occupational lung diseases such as silicosis and asbestosis also show distinctive distribution-

al features (189). Of course, factors in addition to deposition, such as clearance, redistribution, and local sensitivity, also act to determine the distribution of aerosol-induced lung lesions. Precise examination of regional differences requires dissection of the lung after exposure. Stokinger and co-workers (190) examined lobar distribution of aerosol deposition in excised rat lungs; deposition in the upper right lobe occurred at a rate 150 to 175 % faster than that in any other lobe.

Raabe and associates (191) carried out a comprehensive study of deposition in the lung as a function of particle size in hamsters and rats, including the distribution of deposition within the respiratory tract. Their data showed a broad minimum for total deposition (14 to 17 %) at an aerodynamic diameter of 0.52 μm, but a monotonic increase in deposition in the pulmonary compartment with decrease in particle size within the range investigated (0.2 to 3.05 μm). Bronchial deposition (as assayed by amount cleared during 20 h) was also minimal (less than 1 % of the deposited aerosol) for particles 0.52 μm diameter. Interestingly, the relative lobar activities showed that for the whole range of particle size, the apical lobe of the right lung received the largest percentage deposition when compared to its percentage of total lung weight. (The left lung was not separated into pieces.)

In another study of 160 Syrian golden hamsters, Thomas and Raabe (192) exposed one group to a monodisperse aerosol of aerodynamic diameter 1.5 μm and another group to a polydisperse aerosol of median aerodynamic diameter 1.9 μm. Deposition was similar in the 2 groups, but again, right apical lobes consistently contained more activity per unit weight and the right cardiac and right diaphragmatic lobes, less activity per unit weight than the lung as a whole. This disparity increased as the aerodynamic diameter of the particles increased. Morphometric measurements reported in this same study (192) showed that, in both hamsters and rats, the typical path from trachea to terminal bronchioles is shorter for the right apical lobes than for other right lobes.

Yu and Taulbee (193) have developed a refined model of aerosol deposition that takes into account the mechanisms responsible for depositing particles and incorporates the anatomic lung model of Weibel (193a) The predictions of the model are in good agreement with experimental findings, and the model has the flexibility to adjust to different breathing patterns and to predict regional deposition. The model of Yu and Taulbee (193) predicts a minimal total deposition of 10 % for particles close to 0.3 μm in diameter. This minimum is considerably lower than the ICRP value of 30 %, but is more in agreement with recent experimental results, such as those of Heyder and co-workers (187, 194).

A number of investigators have modeled and studied deposition at lung bifurcations. Hot spots develop, where deposition is greater than average by a factor of 5 to 30 (195–199). A shortcoming of all of these studies is that deposition was studied only during inspiratory flow; flow profiles of gas and aerosol are markedly different during expiration.

Even though aerodynamic size is the most important characteristic of an aerosol, some particles, such as those of fibers, flakes, and aggregates, have unusual shapes that preclude predicting aerodynamic behavior from density and geometric appearance. Even if fibers have a sufficiently low aerodynamic diameter to allow penetration to alveoli, they are often deposited by interception, a mechanism independent of their aerodynamic behavior. Models of deposition in the lung predict that the number of straight fibers deposited should be twice the number of irregularly shaped particles deposited (200). Experiments on fiber deposition in rats showed a rapid increase in the total percentage deposition for aerodynamic diameters of 1 to 2.5 μm, with deposition increasing from 30 to 80 % (201).

Hygroscopicity

The addition or removal of water can significantly affect the size of an aerosol particle. Aerosols composed of sodium chloride (NaCl) crystals avidly take up water, so that an aerosol that began as crystals becomes water droplets containing dissolved salt. Cinkotai (202) has discussed the growth of NaCl particles in moist air and has shown that the increase in diameter becomes particularly marked as the relative humidity increases above 95 % and approaches 100 %. At 95 % humidity, a tripling in diameter of NaCl particles can be expected; at 100 % humidity, the size of the NaCl particle can grow without bound. The exact relative humidity within airways is not known, and our understanding of the dynamic growth of inhaled hygroscopic particles in the respiratory tract is still inadequate. Dry atmospheric aerosols have been found to double in diameter when the relative

humidity is increased to 98 % (203). A theoretical study of the growth of dry salt particles of various kinds was undertaken by Ferron (204). He applied his results to the ICRP model and found that, although over-all deposition was little changed, deposition among the various compartments differed markedly from the ICRP recommendations, which were based on the assumption that hygroscopic particles reach their equilibrium size in lung. Ferron's results predict lower nasopharyngeal deposition for the 1- to 10-μm particles, enhanced tracheobronchial deposition for 0.5- to 6-μm particles, and enhanced pulmonary deposition for particles larger than 0.4 μm. For example, tracheobronchial deposition is predicted to be 4 times greater for 2-μm particles than that predicted by the ICRP model. On the one hand, it is clear that the hygroscopic character of some aerosols must be taken into account when attempting to predict how a particle will be deposited. On the other hand, droplets of pure water can evaporate rapidly even under conditions of 100 % humidity because of the increased pressure inside a small droplet caused by surface tension. For example, a 1-μm droplet of water will evaporate within 0.5 s at room temperature even under saturated conditions. A 10-μm droplet will evaporate within approximately 1 min (130).

The lifetime of an aerosol in the ambient atmosphere should also be considered, apart from the behavior of the particles once they are in the lung. The size range typical of aged aerosols is 0.2 to 0.5 μm. Although particles outside this size range may be copiously generated, larger particles precipitate out and smaller ones coagulate. The consequence of coagulation is an increase in the size of the smaller particles, which also serve as condensation nuclei for the absorption of water.

Electric charge

Airborne particles can carry an excess of positive or negative charge acquired either in the process of formation or from the atmosphere. Charged particles discharge with a time constant of approximately 400 s, until they reach equilibrium. At equilibrium, the average number of excess positive or negative charges, q, is given by

$$q = 2.37\ D^{\frac{1}{2}} \qquad [19]$$

where D is the particle diameter, in μm (130).

An electric charge on a particle affects its behavior in 3 ways: (*1*) the particle moves along electric field lines; (*2*) evaporation from and condensation (coagulation) onto the particle are altered; (*3*) the particle is attracted to a neutral surface by the image charge induced. Because negligible electric fields exist within the air spaces of the respiratory system, the first consideration does not apply, although the effects on pulmonary deposition of electric charge on the body have been studied (205). Except for the collection of water, the second process is primarily important in altering the characteristics of the aerosol while it is still outside the respiratory tract. The image-force effect is potentially an important process in enhancing deposition, and its operation in the respiratory tract has been analyzed theoretically by Brock and Marlow (206). Although the analysis predicts that deposition will be enhanced when there are 10 or more charges per particle, experimental studies do not show a conclusive effect. Fry (207) produced aerosols of preformed particles in the size range of 2 to 9 μm by atomization; he found no significant difference between nasal deposition of the aerosol before and after it had passed through a charge neutralizer that decreased the average charge per particle 6- to 8-fold, depending on the diameter. Melandri and co-workers (208) studied the total deposition of particles 0.33, 0.65, and 1.10 μm in diameter that were highly charged by a corona discharge apparatus to 50 to 100 positive charges/particle. Deposition was increased in the range of 15 to 30 %. Because the deposition did not increase with increasing concentration of the aerosol, Melandri and associates (208) attributed the enhanced deposition to image-force effects, rather than self repulsion of the aerosol. Chan and co-workers (209) examined deposition of charged particles in a hollow cast of the human tracheobronchial tree and also found small but significant enhancement of deposition. In conclusion, it appears that, experimentally, electric charge is not an important factor in deposition, unless one is dealing with highly charged, freshly generated particles.

Breathing Pattern

In addition to size of the aerosol, the pattern of ventilation is a key factor in determining the relative importance of the various mechanisms of deposition. Inertial impaction depends on the velocity of the air stream. The effects of diffusion and settling increase with time. Hence,

the breathing pattern influences the effectiveness of mechanisms of the deposition. Inertial impaction is important in upper airways, where velocities are high; diffusion and settling are important in lower airways, where residence time is longer. Minute volume determines the total particle burden that enters the respiratory tract. Hence, even if the deposition fraction is fixed, increasing minute volume results in an increased particle burden for the lung. Dennis (210) has reported that during exercise, the deposition fraction increases with minute volume, particularly for larger particles (1.0 to 3.0 μm); thus, the amount of aerosol deposited in the lung per unit time is doubly increased by the amount presented and by the percentage deposited. An important consideration in aerosol deposition is the airflow profile. As minute volume and, consequently, flow velocity increase, turbulent air flow may penetrate more deeply into the lung. To determine the nature of flow, it is useful to calculate the Reynolds number (Re), which is given by:

$$\mathrm{Re} = \frac{d\rho v}{\eta} \qquad [20]$$

where d is the diameter of the tube; ρ, the density of the gas; v, the flow velocity; η, the viscosity of the gas. For the types of bifurcating tubes found in the respiratory tract, turbulent flow will develop if the Reynolds number exceeds 1,000. During normal viscous (laminar) flow, airflow is most rapid at the center of a tube, so that aerosol-laden air entering the airways is surrounded by a sheath of residual gas that usually contains a lower concentration of aerosol. Compared to the same volumetric laminar flow, turbulent flow causes more mixing within the airstream, and radial transport of aerosol into residual gas and onto the airway walls is greatly accelerated.

In exercising humans, minute volumes can increase to 120 L/min, and this will increase the delivery of aerosol. Even at rest, inspiratory flows are greatly increased during speech (211, 212). The high velocities (2 m/sec) in the main bronchi increase inertial impaction and turbulence. But larger pulmonary volumes in the distal lung mean that distances to surfaces of the lung are increased, so that the fraction of aerosol deposited there may decrease. The ICRP Task Group concluded that, although the absolute amount of aerosol deposited increased with increasing minute volume, the deposition fraction was relatively independent of minute volume, at least within the range 0.75 to 2.0 L/min. Experimenting with 0.5-μm monodisperse particles, Muir and Davies (213) found that the percentage of particles deposited increased linearly with tidal volume and decreased with the square root of breathing frequency. The effect of changes in tidal volume was very small. Later experiments by Davies and associates (214, 215) incorporated the effect of expiratory reserve volume; the results showed that deposition decreased when the resting expiratory level was increased. Palmes and coworkers (91, 117) showed that loss of aerosol from the inspired air was an exponential function of breath-holding time. Loss of aerosol also increased markedly with depth of inhalation. Pavia and co-workers (216) showed that deposition of aerosol was affected by airway obstruction, as gauged by 1-s forced expiratory volume (FEV_1) values; deposition increased by 11 % for every 1-L increase in FEV_1. From an analysis of loss of aerosol in single-breath inhalations, Taulbee and co-workers (217) concluded that mixing of aerosol particles 0.5 to 1.0 μm in diameter with reserve air is important only in the alveolar region of the lung, where the interface is spread over the greatest surface area. Otherwise, aerosol delivery follows convective air motions very closely.

Experiments measuring the effect of breathing pattern on the distribution of aerosol deposition are few. Foord and co-workers (218) administered monodisperse particles in the size range of 2.5 to 7.5 μm to mouth-breathing subjects to study regional deposition measured by comparing initial retention to 24-h retention. Regional deposition, compared to the sum of pulmonary and tracheobronchial deposition, was a monotonically decreasing function of the impaction parameter, D^2F, where D is the aerodynamic diameter of the particle, and F is average inspiratory flow.

Valberg and associates (132) have reported experiments in which excised dog lungs were ventilated with radioactive aerosol according to various breathing patterns. The excised dog lungs were ventilated in a lucite box while positioned horizontally with the dorsal aspect supported by a layer of saline and the trachea attached to a cannula leading out of the box. Negative pressure inside the box was varied in a manner dictated by a waveform generator. A wedge spirometer was used to determine both pressure-volume curves and the desired ventilatory patterns. The breathing patterns tested

were called "slow deep" i.e., a tidal volume (V_T) of 40 % of the total lung capacity (TLC) and a breathing frequency (f) of 12 breaths/min, "rapid, shallow" (V_T = 15 % TLC; f = 84 breaths/min), and "exercising" (V_T = 30 % TLC; f = 30 breaths/min). The functional residual capacity was maintained at 30 to 40 % TLC.

Sulfur-colloid particles labeled with ^{99m}Tc were aerosolized using a 5-MHz ultrasonic nebulizer, and the initially 1.5 to 2.0-μm diameter droplets were dried to yield an aerosol that was log normally distributed with an activity median aerodynamic diameter of 0.4 μm and a GSD of 1.35. After 0.5-h exposure, the lungs were fully expanded to a pressure of 35 cm H_2O and dried in this state. After the rigid lungs were sliced transversely at 1-cm intervals, each slice was weighed and photographed. The distribution of retained radioactive aerosol was determined in 3 ways: (*1*) by gamma camera, (*2*) by autoradiography, and (*3*) by further dissection of each slice into 1-cm^3 pieces that were then scored for airway content, assayed for radioactivity, and weighed.

Deposition efficiency was 42 % for slow deep breathing, 21 % for the exercising pattern of breathing, and 9 % for rapid, shallow breathing. To analyze distribution of deposition for each region of lung, regional deposition was expressed as an evenness index, as previously described (155). The evenness indices showed that, during slow deep breathing, the parenchyma received more than an average share of deposition and large airways received less than an average share. In the exercising pattern of breathing, deposition in the large airways increased. During panting (rapid, shallow breathing), large airways received more deposited activity per unit volume than parenchyma.

When the lung was cut into pieces, each piece was given a score representing the proportion of its volume estimated to be airway. These classifications were: 3, for more than 50 % airway; 2, 10 to 50 % airway; 1, less than 10% airway; 0, no visible airway. When the radioactivity was normalized with respect to weight, the over-all low evenness index of airways reflected their large contribution to the weight of a dried lung. The evenness index was better correlated with the type of tissue sample than with the breathing pattern. However, pieces containing airways still had more deposition for the exercising and panting patterns of breathing than for slow deep breathing.

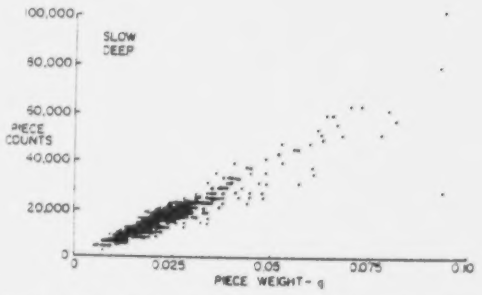


Fig. 14. *A*. Activity of individual (approximately 1-cm^3) pieces of dog lung plotted as a function of their weight for a lung exposed to radioactive aerosol while ventilated in a slow deep pattern.

The data discussed reflect the mean activity of pieces of lung from the various categories. For the parenchyma (< 10 % airways), the mean activity was approximately the same, regardless of the breathing pattern. Variation in activity among pieces was also examined. The 2 graphs in figure 14 show the weight of each piece plotted as a function of its activity. For slow deep breathing (figure 14A), weight and activity correlated well, i.e., the data for all pieces of lung were clustered closely around a single evenness index. For the exercising pattern of breathing (figure 14B), deposition of aerosol varied considerably from piece to piece, even for pieces of the same weight. Thus, the evenness indices of individual pieces were quite heterogeneous. Such shifts could change the physiologic effect of the deposited aerosol considerably, even if total pulmonary deposition remained unchanged.

Mouth versus Nose Breathing

In both animals and humans there are important implications for particle deposition and clearance patterns in the choice of pathway to

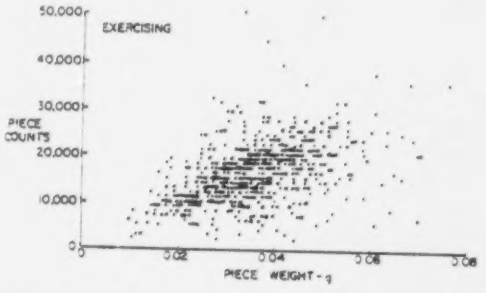


Fig. 14. *B*. The same plot as *A* for a lung exposed to aerosol while ventilated in a more rapid, shallow breathing pattern than that in A.

the trachea. The nasal and oral routes have very different properties, and shifts from one pathway to the other can be of major significance. Abundant evidence indicates that significant fractions of inhaled particles and gases can be deposited in the nose and pharynx. An excellent review of particle deposition in that region is that of Hounam and Morgan (219). A discussion of gas uptake by the nose is also available (220). Deposition and clearance of particles in the head during nose breathing have been extensively studied by Pattle (221), Hounam and co-workers (222), Lippmann (223), and Martens and Jacobi (224). Collectively, these reports indicate that important quantities of particles larger than 5.0 μm are deposited in the nasopharyngeal region. There are large individual variations, depending on the structure and aerodynamic properties of the nose. Hounam and associates (222) showed a clear correlation between the percentage of inspired particles deposited in the nose and the resistance to flow.

The ability of the nose to remove large particles and highly soluble gases from inhaled air forms an important element in our system of respiratory defense mechanisms and reflects specific anatomic features. Narrow cross sections and the resulting high linear velocities, sharp bends, and nasal hairs all conspire to promote impaction of aerosols (225). The nose, at least at modest flows, warms and humidifies the air. When the particles are hygroscopic, both the size of the particles and collection efficiency may increase.

In every person, the nasal route is more efficient at removing particles from inspired air than is the oral route. By bypassing the nose, mouth breathers deposit more particles in their lungs and may concomitantly increase the risk of pulmonary damage. Mouth breathing is common under conditions of heavy work, during speech (211, 212), and in those who have impaired nasal conductance. This may result in markedly increased exposure of the airways and alveoli to large particles. Thus, with exercise, the amount of aerosol deposited in the lungs may increase in excess of that predicted by the increased ventilation. It should also be noted that a tracheal tube used for controlled ventilation or for airway protection effectively bypasses the upper airways. Thus, infectious particles and other aerosols of all kinds have greater access to the lower respiratory tract.

The nose may be injured in the course of protecting the lower respiratory tract. Although clearance from the nose is rapid and, hence, residence times for particles are generally short, it does not follow that retention is unimportant. Evidence from studies of occupational diseases suggests that particles deposited in the nasal passages can be retained long enough to produce biologic responses.

Some responses to particles deposited in the nose and pharynx are transitory and reversible. Inhalation allergies to industrial dusts originating from grains, textiles, molds, and so on, and irritation from gases, fumes, dusts, and smokes have been described repeatedly. Chronic irritation of the nasal mucosal membranes can also result in irreversible damage. Perforations of the nasal septum have been noted in workers after prolonged exposures to chromates, arsenic, cement, mercury fulminate, sodium fluoride, chlorine, and lime (226).

More important, neoplasms have also been associated with retention of particles in the nose. Although nasal cancers are rare in the general population, their incidence has increased among various occupational groups. Workers inhaling wood dusts during the manufacture of furniture show an increased incidence of nasal cavity and sinus cancer; the predominant type is adenocarcinoma (227–230). Persons employed in the manufacture of leather shoes and boots are also at substantial risk of developing intranasal cancers. The causative factor is believed to be associated with particles of dust from leather, fiberboard, rubber, or cork (228). An association between exposure to asbestos and carcinoma of the head and neck has been reported (231). Finally, increased risk of nasal cancers has been noted in nickel workers (232, 233), in persons engaged in the production of chromates (234), and in workers who paint watch dials with radium (235).

Species Differences

A major challenge in modern toxicology is the problem of extrapolating findings from one species to another and from animals to humans. Although many different species have been used to study deposition of aerosols, no complete and systematic description of the differences among commonly used laboratory animals is available. It is difficult to abstract a comprehensive description of species differences from the literature because so many different kinds of animals and aerosols have been used in various combi-

nations. A few theoretical and experimental contributions have been made, but the problem is far from solved. Palm and co-workers (236) studied retention of dust in the lungs of guinea pigs and small monkeys; the percentage of alveolar retention as a function of particle size was substantially the same as that found earlier in humans. Friedlander (237) made use of dimensional analysis to investigate deposition of particles in the lower lung. Kliment (238) identified several of the important dimensionless groups of physiologic variables that appear in aerosol deposition problems and predicted the degree to which these groups of variables are the same in rats, guinea pigs, rabbits, and humans. More recently, Stauffer (239) has used dimensional analysis to predict that the probability of deposition of inhaled aerosols should be the same for different animals in the case of sedimentation or mixing-dominated deposition, but should scale as (body weight)$^{-0.1}$ for diffusion-dominated deposition. The predictions of McMahon and associates (240) differ from those of Stauffer (239) and suggest that diffusion-dominated deposition is independent of body weight.

McMahon and associates (240) suggest specifying each of the physical mechanisms of particle deposition by identifying the controlling dimensionless group. For example, in the particle impaction problem, the collection efficiency, E (the percentage of particles entering that is actually deposited) increases as the Stokes' number, St, increases. The Stokes' number is the ratio of the stop distance of a particle to the characteristic dimension of the system (see equation 16). Thus, the free stream velocity, u, the particle density, ρ, the square of the particle radius, r, the viscosity of the air, η, and the characteristic dimension of the collector, R, all enter the product. Similarly, the Froude number, v, which contains the ratio of the particle sedimentation velocity and the through-flow velocity, governs the sedimentation of particles in the nose, pharynx, and large airways during quiet breathing. Diffusion of small particles to the walls of the alveoli increases when the dimensionless diffusion time, DT/a^2, increases, where D is the diffusion coefficient, which depends directly on temperature and inversely on air viscosity and particle radius, T is the breath period, and a is the alveolar diameter.

If each of the dimensionless groups listed previously varied with the animal's body weight, understanding the scaling rules for total deposition of particles in the lung would be complicated and difficult. Actually, several of the dimensionless groups are independent of body weight. Although many important physiologic parameters such as breath period and ventilation can be expected to change as animals of dissimilar body weight are compared, competing effects sometimes cancel one another in the controlling dimensionless groups, so that the collection efficiency of any part of the lung may be independent of body size for the same size of aerosol.

McMahon and co-workers (240) tested these ideas with a "Noah's Ark" approach. They simultaneously exposed 6 different species to the same 0.78-μm aerosol of gold-198 and compared both total deposition and site of deposition among species. These results were plotted against body weight on logarithmic scales to obtain power-law equations of the type that have been used successfully to summarize other respiratory data (241). These regressions were interpreted in the context of scaling theories already developed by McMahon (242) that predict a change in body and organ geometry as body weight changes among species. Power-law equations and their interpretation constitute the scaling laws necessary to predict the collection efficiency of upper airways and alveoli from one species to another. When the total amount of aerosol deposited was divided by the animal's body weight, it was found that the smaller animals had received more particles/g than the larger ones. However, the collection efficiencies (the fraction of inhaled aerosol retained) of both the lung alone and the lung, nose, pharynx, and airway taken together were substantially independent of body size. A reasonable conclusion is that the collection efficiency of the lung alone and the collection efficiency of the entire respiratory tract are, as expected, substantially independent of body size for aerosols of submicrometer diameter.

It is important to remember that the selection of the species to be exposed will influence the resulting dose to the lung. Different species breathing the same aerosol do not receive identical doses. Individual variability is also large. Variations among individual animals of the same species may exceed 100 %. These differences in deposition might, in large part, be due to the variations in nasal geometry known to be present in otherwise equivalent animals and to different levels of activity.

It is obvious that animals differ among them-

selves and from humans not only in lung size, but also in respiratory rate and airway branching patterns. The human bronchial tree is more symmetric in its branching pattern than that of dogs, cats, rodents, or horses. In addition, the human nose is far less efficient as a particle filter than that of the dog. It is essential to realize that different rates of clearance among species can influence retention and, therefore, the total dose retained by the lung. Thomas (243) has developed a model describing the kinetics of clearance of inhaled particles in the respiratory tract of mice, rats, and dogs.

Disease

Deposition of aerosols is markedly altered by significant pathologic changes in airways and parenchyma. Lippmann and associates (244) have reported dramatically increased bronchial deposition in subjects with chronic bronchitis and asthma; these increases may change with different phases of the disease (245). Most abnormal states appear to enhance airway deposition at the expense of pulmonary deposition. For example, Lourenço and co-workers (246) have shown that the deposition of 2-μm particles in patients with bronchiectasis is frequently more central than that in normal subjects.

Parenchymal involvement and airway obstruction are often associated with decreased or absent deposition in the alveoli. Taplin and coworkers (93) have discussed how partial or complete airway obstruction in bronchitis, lung cancer, emphysema, fibrosis, and atelectasis may decrease or eliminate deposition of particles in some parts of the lung. Jakab and Green (247) studied the retention of radiolabeled bacteria in normal mice and mice with pneumonia due to a previous infection with Sendai virus. The retention of bacteria was compared in consolidated and nonconsolidated areas of the lungs. Ninety per cent of the retained bacteria were deposited in the nonconsolidated areas, whereas only 10 % were found in consolidated areas of similar volume.

The mechanisms responsible for these alterations in the pattern of deposition are numerous, complex, and interactive. Airway obstruction diverts inspired flow to unobstructed airways. Decreased cross section of the airways increases linear velocity and turbulence, thereby enhancing inertial impaction and central deposition. Many diseases are also associated with alterations in alveolar dimensions that may alter the rate of deposition of particles in parenchyma. Breathing patterns may also be drastically altered.

Partial or complete obstruction of the airways due to excessive secretions, bronchospasm, or tumors will significantly decrease delivery of therapeutic aerosol to sections of the lung peripheral to the obstruction. Aerosols can be used to determine ventilation defects in the lung. Pavia and co-workers (248) found that the depth of deposition in the lung is positively correlated with FEV_1, so that in patients with airway obstruction, peripheral deposition is decreased. These same workers (248) concluded that to enhance peripheral deposition of therapeutic aerosols, a person with obstructive lung disease should inhale slowly and hold his or her breath at the peak of inspiration. In addition, the particles should be inhaled from a container in which the particles lose the velocity imparted to them during generation. Particles should be less than 5 μm in aerodynamic diameter to penetrate beyond the pharynx and major airways.

It is important to recognize that disease may affect not only mechanisms of deposition, but also mechanisms of clearance. To the extent that clearance is decreased, retention will be correspondingly greater. Because this review focuses on deposition, the effects of disease on clearance will not be discussed; however, their importance is frequently greater than that of deposition per se. Sometimes, disease compromises clearance and increases retention. In other instances, disease acts to narrow the cross section of the airways and favors more central deposition. Because particles are deposited closer to the mouth, they may be cleared at a more rapid rate from the respiratory tract.

Other Factors

Exercise. Increases in physical activity with associated increases in ventilation are also important determinants of the total dose and distribution of deposited inhaled particles. Exercise can result in changes from the nasal pathway to the oral pathway (see *Mouth versus Nose Breathing*) and changes in tidal volume, breathing frequency, and lung volume (see *Breathing Pattern*). In fact, most relevant human studies have focused on the effects of changing breathing patterns, rather than exercise. These investigations commonly compare the particle contents of inhaled and exhaled air to determine deposition. Changes in tidal volume, rate of breathing, minute volume, and expira-

tory reserve volume, all of which may change with exercise, have been shown to influence the number of particles deposited.

Few animal studies have investigated the effects of exercise or increased ventilation on deposition. The effect of physical activity on the inhalation toxicity of a paraoxon aerosol (0.9 μm in diameter) has been examined (249). The toxicity was increased 2.7 times in exercising rats, which had minute ventilations approximately twice those of the control animals. In a study of deposition of labeled tobacco smoke in the respiratory tract, it was noted that hamsters breathing through oral cannulas had 2 to 3 times the lung deposition of normally breathing animals (250).

Recently, studies carried out in our laboratory (Harbison M and Brain JD: Unpublished data) have investigated the deposition of radioactive aerosols in exercising and resting hamsters. Using a small animal treadmill placed in an exposure chamber, we found that as O_2 consumption increased from 1 to 6 ml of O_2/100 g of body weight/min, the retention of particles increased by a factor of approximately 9. A plot of O_2 consumption and retention fell in a straight line, with surprisingly little scatter. In the same animals, the collection efficiency (the fraction of inhaled particles actually deposited in the lung) increased from approximately 8 to 13 % within the same range of O_2 consumption. In most animals, a gradient of retained particles was present, with more in apical slices ("apical" here designating anatomic location and not necessarily gravitational position) than basal slices.

Regional differences and gravity. As just noted, we have observed regional differences in the deposition of aerosols in both resting and exercising hamsters. Previously published experiments (155) had shown that rats and hamsters received greater deposition/g of tissue in the apical than basal portions of the lung. Earlier studies in anesthetized dogs exposed to radioactive aerosols (Brain JD: unpublished data) had revealed that the uppermost segments of the lungs received as much as 20 times the deposition/g of lung as did the lowermost segments (figure 15). This observation appeared to contradict the classic dogma that most of the ventilation goes to dependent portions of the lung, where alveolar volumes are lower and, hence, compliance is greater. The results, therefore, suggest that the regional distribution of inspired aerosol may be different from that of inspired gas and/or there are significant differences in regional collection efficiency. Because we suspected that gravity and regional differences in alveolar and airway dimensions and transpulmonary pressure were involved, we conducted a series of experiments (251) to study the effects of body position and, by implication, the effects of gravity on aerosol deposition in the lungs of small animals. We exposed groups of 15 animals, with 5 in each of 3 positions: standing, prone, and inverted. All animals were awake. The aerosol had an activity median aerodynamic diameter of 0.78 μm. As in the earlier experiments, deposition was greater in the apex than in the base of the lung in all animals, regardless of body position. The experiments were repeated with a mixture of 20 % O_2 and 80 % helium and a mixture of 20 % O_2 and 80 % SF_6. There were small differences and considerable individual variability, but in general, deposition was greater in the apex than the base of the lung. Thus, in small animals, there are important apex-to-base gradients in aerosol deposition that persist in the face of changing body position and gas properties; the mechanisms involved are still being examined.

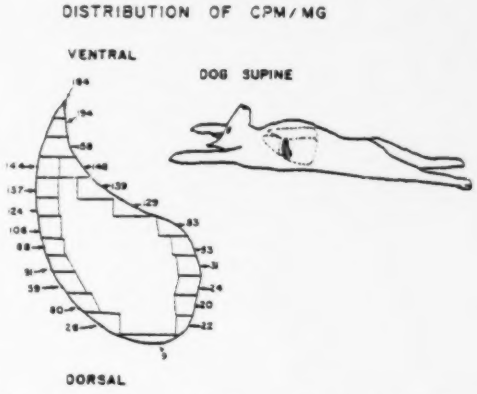


Fig. 15. Distribution of radioactivity in a dried lung slice obtained from a spontaneously breathing, anesthetized dog. The dog was killed 3 h after a 40-min exposure to ^{99m}Tc labeled aerosol. The particle size of the aerosol was 2.4 μm, with a geometric standard deviation of 1.8. Note the dramatic difference in the concentration of the aerosol between the top and bottom of the lung.

These regional differences were observed in a 1-g field, but aerosol deposition and the distribution of deposited particles would change if gravity were increased or decreased. Hoffman and Billingham (252) studied the deposition of 2.02-μm particles at 0.5, 1.0, and 2.0 *g*; total

deposition increased linearly with increasing g within the range studied. No estimates of the distribution of particle deposition were obtained, although this probably changed as well.

Age. Most animal and human studies have been performed in adults; yet there is growing concern about the effects of cigarette smoke and air pollutants on the lungs of children. Infectious aerosols have always represented a major threat to the health of children. Although the topic is of considerable interest, only fragmentary and sometimes conflicting data exist.

Muir (253) estimated that the filtration efficiency of airways in the newborn is similar to that of the adult. However, when Sikov and Mahlum (254) studied the deposition of 1- to 3-μm aerosols in newborn (1-day-old) and adult rats, they found lower deposition fractions in the lungs of the immature animals. This might have been due to greater deposition of larger particles in the smaller upper airways of the newborn rats. There is a need for more research in this area.

Conclusions

Emphysema, bronchitis, pneumoconiosis, neoplasms, and infectious disease all may be consequent to inhalation of noxious particles, and the severity of resultant disease may be influenced by the number and distribution of particles deposited. But therapeutic aerosols may alleviate disease if they can be delivered to appropriate sites within the respiratory tract. The importance of aerosols as probes of lung anatomy and/or function is also being recognized. We believe that new knowledge about aerosols can yield insights into the factors that predispose human populations to pulmonary disease arising from exposure to inhaled particles. Furthermore, we believe these ideas will lead to better scientific strategies for inhalation therapy involving accurate placement of therapeutic aerosols, and to improved use of aerosols as tools in studies of pulmonary physiologic and pathophysiologic features.

It is important to remember that exposure to an aerosol does not adequately define the dose produced nor does it specify the anatomic distribution of that dose. Regardless of the aerosol exposure problem being studied, it is essential to quantify the dose. Investment in a thorough description of the location of aerosols in the respiratory tract will be rewarded, because improved understanding of the effects of aerosols stems from the recognition of factors important to their uptake and fate. Published papers dealing with aerosols should include information about the mass concentration, size distribution, and breathing pattern used as well as estimates or measurement of aerosol retention. Also needed is research into better ways of generating and delivering aerosols in order to obtain more precise dose localization. Finally, more studies are required to demonstrate the effectiveness of aerosol therapy. Many current strategies are both expensive and of questionable value. At the policy level, we need better control of public exposures to toxic aerosols to prevent respiratory disease.

References

1. Davies CN, ed. Inhaled Particles and Vapours. New York: Pergamon Press, 1961.
2. Davies CN, ed. Inhaled Particles and Vapours II. New York: Pergamon Press, 1967.
3. Walton WH, ed. Inhaled Particles III. Surrey, England: Unwin Brothers, 1971.
4. Walton WH, ed. Inhaled Particles IV. New York: Pergamon Press, 1977.
5. Aharonson EF, Ben-David A, Klingberg MA. Air Pollution and the Lung. New York: John Wiley and Sons, 1976.
6. Altshuler B, Yarmus L, Palmes ED, Nelson N. Aerosol deposition in the human respiratory tract. Arch Ind Health 1957; 15:293–303.
7. Brain JD, Proctor DF, Reid L, eds. Respiratory Defense Mechanisms, vol. 5. In: Lenfant C, chief ed, Lung biology in health and disease. New York: Marcel Dekker, 1977.
8. Brain JD, Valberg PA. Models of lung retention based on the report of the ICRP Task Group. Arch Environ Health 1974; 28:1–11.
9. Hatch TF, Gross P. Pulmonary deposition and retention of inhaled aerosols. New York: Academic Press, 1964.
10. Lippmann M. Regional deposition of particles in the human respiratory tract. In: Lee DHK, Murphy SD, eds. Handbook of physiology, sect. 9, Reactions to environmental agents. Bethesda: American Physiological Society, 1977:213–32.
11. Stuart BO. Deposition of inhaled aerosols. Arch Intern Med 1973; 131:60–73.
12. Morrow PE, Chairman, Task Group on Lung Dynamics. Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys 1966; 12:173–207.
13. Palmes ED, Lippmann M. Influence of respiratory air space dimensions on aerosol deposition. In: Walton WH, ed. Inhaled Particles IV. Part I. New York: Pergamon Press, 1977: 127–36.

14. Palmes ED. Measurement of pulmonary airspaces using aerosols. Arch Intern Med 1973; 131:76–9.
15. Albert RE, Lippmann M, Peterson HT Jr, Berger J, Sanborn K, Bohning D. Bronchial deposition and clearance of aerosols. Arch Intern Med 1973; 131:115–27.
16. Albert RE, Arnett LC. Clearance of radioactive dust from the human lung. Arch Ind Health 1955; 12:99–106.
17. Lippmann M, Albert RE, Yeates DB, Morrow PE. Effects of inhaled particles on man and animals. I. Deposition, retention and clearance. National Research Council, 1977.
18. Morrow PE. Models for the study of particle retention and elimination in the lung. In: Inhalation Carcinogenesis. USAEC Symposium, Series No. 8, 1970:103–115.
19. Duguid JB. The size and duration of air-carriage of respiratory droplets and droplet nuclei. J Hyg (London) 1946; 44:471–9.
20. Loudon RG, Roberts RM. Droplet expulsion from the respiratory tract. Am Rev Respir Dis 1967; 95:435–42.
21. Wells WF. On air-borne infection. II. Droplets and droplet nuclei. Am J Hyg 1934; 20:611–18.
22. Edmonds RL, ed. Aerobiology: the ecological systems approach. Stroudsburg, Pa.: Dowden, Hutchison and Ross, 1979.
23. Riley RL. Airborne infection. Am J Med 1974; 57:466–75.
24. McDermott W. Conference on airborne infection. Bacteriol Rev 1961; 25:173–94.
25. Top FH. Communicable and infectious diseases. St. Louis: CV Mosby Co., 1964.
26. Reinarz JA. Nosocomial infections. Clin Symp 1978; 30:1–32.
27. Leedom JM, Loosli CG. Airborne pathogens in the indoor environment with special reference to nosocomial (hospital) infection. In: Edmonds RL, ed, Aerobiology: the ecological systems approach. Stroudsburg, Pa.: Dowden, Hutchison and Ross 1979:208–210.
28. Phillips I, Spencer G. Pseudomonas aeruginosa cross-infection due to contaminated respiratory apparatus. Lancet 1965:1325–7.
29. Reinarz JA, Pierce AK, Mays BB, Sanford JP. The potential role of inhalation therapy equipment in nosocomial pulmonary infections. J Clin Invest 1965; 44:831–41.
30. Muir DCF. Airborne allergens. In: Muir DCF, ed, Clinical aspects of inhaled particles. Philadelphia: Davis, 1972:54–69.
31. Tees EO, Milner FH. The allergens of asthma. Acta Allergol (Kbh) 1962; 17:536–46.
32. Wilkin-Jensen K. The diagnosis of allergic diseases. In: Jamar JM, ed, International textbook of allergy. Springfield, Ill.: Charles C Thomas, 1959:121–41.
33. Patterson R. Aeroallergens. In: Patterson R, ed, Allergic diseases: diagnosis and management. New York: JB Lippincott 1972.
34. Durham OC. The pollen content of the air in North America. J Allergy 1935; 6:128–49.
35. Jacobson AR, Morris SC. The primary air pollutants—viable particulates: their occurrence, sources and effects. In: Stern AC, ed, Air pollution, vol. I, Air pollutants: their transformation and transport. New York: Academic Press, 1976:169–96.
36. Hobday JD, Townley RG. Deposition of inhaled pollen grains in the lower respiratory tract of the guinea pig. J Allergy Clin Immunol 1971; 48:254–6.
37. Busse WW, Reed CE, Hoehne JH. Where is the allergic reaction in ragweed asthma? II. Demonstration of ragweed antigen in airborne particles smaller than pollen. J Allergy Clin Immunol 1972; 50:289–93.
38. Lanza AJ, ed. The pneumoconioses. New York: Grune and Stratton, 1963.
39. Eckardt RE. Occupational cancer: methods of epidemiological study. Arch of Ind Health 1959; 19:335–8.
40. Walsh PJ, Hamrick PE. Radioactive materials: determinants of dose to the respiratory tract. In: Falk HL, Murphy SD, eds, Handbook of Physiology, sec. 9; Reactions to environmental agents. Bethesda: American Physiological Society, 1977:233–42.
41. Krey PW, Leifer R, Benson WK, Dietz LA, Hendrikson HC, Coluzza JL. Atmospheric burnup of the Cosmos-954 reactor. Science 1979; 205:583–5.
42. Peller S. Lung cancer among mine workers in Joachimsthal. Hum Biol 1939; 11:130–5.
43. Seltser R. Lung cancer and uranium mining. Arch Environ Health 1965; 10:923–36.
44. Lundin FE Jr, Lloyd JW, Smith EN, Archer VE, Haladay DA. Mortality of uranium miners in relation to radiation exposure, hard-rock mining, and cigarette smoking—1950 through 1967. Health Phys 1969; 16:571–8.
45. DeVilliers AJ, Windish JP. Lung cancer in a fluorspar mining community. I. Radiation, dust and mortality experience. Br J Ind Med 1964; 21:94–109.
46. Eisenbud M, Petrow HG. Radioactivity in the atmospheric effluents of power plants that use fossil fuels. Science 1964; 144:288–9.
47. Bradshaw RL, Setter LR. Radioactive particles in the atmosphere at Cincinnati, Ohio. Public Health Rep 1958; 73:431–8.
48. Lockhart LB Jr, Patterson RL Jr, Saunders AW Jr. The size distribution of radioactive atmospheric aerosols. J Geophys Res 1965; 70: 6033–5.
49. Scalf MR, Ledbetter JO. The solubility of airborne radioactive particles. Am Ind Hyg Assoc J 1965; 26:161–4.

50. Mamuro T, Matsunami T, Maki N. Solubility of fallout particles. Health Phys 1965; 11:316–21.
51. First MW. Aerosols in nature. Arch Intern Med 1973; 131:24–32.
52. Subcommittee on Airborne Particles, National Research Council. Aerosols: characteristics, behavior and measurement. In: Airborne particles. Baltimore: University Park Press, 1979: 1–19.
53. Hidy GM. The dynamics of aerosols in the lower troposphere. In: Mercer TT, Morrow PE, Stober W, eds. Assessment of airborne particles. Springfield, Ill.: Charles C Thomas, 1971: 81–115.
54. Air Conservation Commission AAAS. Report of the AAAS Air Conservation Commission. Washington DC: American Association for the advancement of Science, 1965.
55. Fanger PO, Valbjorn K, eds. Indoor climate. Copenhagen: Danish Building Research Institute, 1979.
56. Willecke K, Whitby KT. Atmospheric aerosols: size distribution interpretation. J Air Pollut Control Assoc 1975; 25:529–34.
57. Fennelly PF. The origin and influence of airborne particulates. Am Scientist 1976; 64:46–56.
58. Corn M, Montgomery TL, Reitz RJ. Atmospheric particulates: specific surface areas and densities. Science 1968; 159:1350–51.
59. Schrenk HH, Heimann H, Clayton GD, Gafafer WM, Wexler H. Air pollution in Donora, Pa. Epidemiology of the unusual smog episode of October, 1948. Public Health Bull no. 306, 1949.
60. Ministry of Health. Mortality and morbidity during the London fog of December 1952. London, 1954.
61. Dean G. Lung cancer among white South Africans. Br Med J 1959; 2:852–7.
62. Reid DD. Environmental factors in respiratory disease. Lancet 1958; 1:1289–94.
63. Doerner AA. Air pollution and chronic lung disease. Ann Allergy 1965; 23:475–83.
64. Ferris BG. Health effects of exposure to low levels of regulated air pollutants: a critical review. Air Pollut Control Assoc J 1978; 28:482–97.
65. Lave LB, Seskin EP. Air pollution and human health. Science 1970; 169:723–33.
66. Mokler BV, Henderson TR. Characterization of aerosolized consumer products. In: Inhalation Toxicology Research Institute annual report. Albuquerque, N.M.: Lovelace Biomedical and Environmental Research Institute, USDE, NTIS 1974:323–3.
67. Frank R. Are aerosol sprays hazardous? Am Rev Respir Dis 1975; 112:485–9.
68. Gowdy JM, Wagstaff MJ. Pulmonary infiltration due to aerosol thesaurosis: a survey of hairdressers. Arch Environ Health 1972; 25: 101–8.
69. Zuskin E, Bouhuys A. Acute airway responses to hair spray preparations. N Engl J Med 1974; 290:660–3.
70. Carter WL, Hasegawa I. Fixation of tobacco smoke aerosols for size distribution studies. J Coll Interf Sci 1975; 53:134–41.
71. Hinds WC. Size characteristics of cigarette smoke. Am Ind Hyg Assoc J 1978; 39:48–54.
72. Hoegg UR. Cigarette smoke in closed places. Environ Health Perspect 1972; 2:117–28.
73. U.S. Public Health Service. Surgeon General's report on smoking and health, 1979: (DHEW publication no. [PHS]79–50066).
74. U.S. Public Health Service. Surgeon General's report on Smoking and Health, 1972:141–150. (DHEW publication no. [HSM] 72–7516).
75. Little JB, Kennedy AR, McGandy RB. Lung cancer induced in hamsters by low doses of alpha radiation from polonium-210. Science 1975; 188:737–8.
76. Radford EP, Hunt VR. Cigarettes and polonium-210. Science 1964; 144:366–7.
77. International Agency for Research on Cancer. Cadmium and inorganic compounds. In: World Health Organization IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Lyon: IARC, 1973:74–94.
78. Szadkowski D, Schultze H, Schaller K-H, Lehnert G. On the ecological importance of the content in heavy metal of cigarette smoke. Arch Hyg 1969; 153:1–8.
79. Doll R. Cancers related to smoking. In: Richards RG, Proceedings of Second World Conference on smoking and health. London: Pittman Medical, 1971:10–25.
80. Severson RF, Snook ME, Akin FJ, Chortyk OT. Correlation of biological activity with PAH content of tobacco smoke fractions. In: Jones PW, Frendenthal RI, eds, Carcinogenesis vol. 3. Polynuclear aromatic hydrocarbons. New York: Raven Press, 1978:115–30.
81. Falk HL. Chemical agents in cigarette smoke. In: Falk HL, Murphy SD, eds, Handbook of physiology, sec. 9, Reactions to environmental agents. Bethesda: American Physiological Society, 1977:199–211.
82. U.S. Public Health Service. Surgeon General's report on smoking and health, 1964. (DHEW Publication no. 1103).
83. Spector SL, Farr RS. A comparison of methacholine and histamine inhalation in asthmatics. J Allergy Clin Immunol 1975; 56:308–16.
84. Lockey RF, Bukantz SC. Diagnostic tests and hypersensitization therapy in asthma. In: Weiss EB, Segal MS, eds, Bronchial asthma. Boston: Little Brown, 1976:613–38.
85. Spector SL, Farr RS. Bronchial provocation tests. In: Weiss EB, Segal MS, eds, Bronchial asthma. Boston: Little Brown 1976:639–62.

86. Altshuler B. The role of the mixing of intrapulmonary gas flow in the deposition of aerosol. In: Davies CN, ed. Inhaled particles and vapours. London: Pergamon Press, 1961:47–54.
87. Altshuler B, Palmes ED, Yarmus L, Nelson N. Intrapulmonary mixing of gases studied with aerosols. J Appl Physiol 1969; 14:321–7.
88. Heyder J, Davies CN. The breathing of half micron aerosols. III. Dispersion of particles in the respiratory tract. J Aerosol Sci 1971; 2:437–452.
89. Muir DCF. Distribution of aerosol particles in exhaled air. J Appl Physiol 1967; 23:210–14.
90. Palmes ED, Altshuler B, Nelson N. Deposition of aerosols in the human respiratory tract during breath holding. In: Davies CN, ed. Inhaled particles and vapors II. New York: Pergamon Press, 1965; 339–49.
91. Palmes ED, Goldring RM, Wang CS, Altshuler B. Effect of chronic obstructive pulmonary disease on rate of deposition of aerosols in the lung during breath holding. In: Walton WH, ed. Inhaled Particles III. Surrey: Unwin Bros, 1971:123–30.
92. Nadel JA, Wolfe WG, Graf PD, *et al*. Powdered tantalum: a new contrast medium for roentgenographic examination of human airways. N Engl J Med 1970; 283:281–6.
93. Taplin GV, Poe ND, Dore EK, Greenberg A, Isawa T. Radioaerosol inhalation scanning. In: Gibson AJ, Pulmonary investigations with radionuclides. Smoals WM, eds, Springfield, Ill.: Charles C Thomas, 1970:296–317.
94. Nadel JA, Wolfe WG, Graf PD. Powdered tantalum as a medium for bronchography in canine and human lungs. Invest Radiol 1968; 3:229–38.
95. Jenne JW. The clinical pharmacology of bronchodilators. Basics of RD 1977; 6:1–6.
96. Innes IR, Nickerson M. Norepinephrine, epinephrine, and the sympathomimetic amines. In: Goodman LS, Gilman A, eds, The pharmacologic basis of therapeutics. New York: Macmillan, 1975; 477–513.
97. Stewart BN, Block AJ. A trial of aerosolized theophylline in relieving bronchospasm. Chest 1976; 69:718–21.
98. Klock LE. A comparative study of atropine sulfate and isoproterenol hydrochloride in chronic bronchitis. Am Rev Respir Dis 1975; 112: 371–76.
99. Altounyan REC. Inhibition of experimental asthma by a new compound-disodium cromoglycate, "Intal." Acta Allerg 1967; 22:487.
100. Irani FA, Jones NL, Gent M, Newhouse MT. Evaluation of disodium cromoglycate in intrinsic and extrinsic asthma. Am Rev Respir Dis 1972; 106:179–85.
101. Campbell IA. Inhaled corticosteroids compared with oral prednisone in patients starting long term corticosteroid therapy for asthma: a controlled trial by the British Thoracic and Tuberculosis Association. Lancet 1975; 2:469–73.
102. Christoforidis AJ, Tomashefski JF, Mitchell RI. Use of an ultrasonic nebulizer for the application of oropharyngeal, laryngeal and tracheobronchial anesthesia. Chest 1971; 59:629–33.
103. Wanner A, Amikam B, Sackner MA. A technique for bedside bronchofibroscopy. Chest 1972; 61.287–8.
104. Egan DF. Aerosol and humidity therapy. In: Fundamentals of respiratory therapy. St. Louis: CV Mosby, 1973:213–68.
105. Wolfsdorf J, Swift DL, Avery ME. Mist therapy reconsidered: an evaluation of the respiratory deposition of labelled water aerosols produced by jet and ultrasonic nebulizers. Pediatrics 1969; 43:799–808.
106. Walkenhorst W, Dautrebande L. Experimental observations on various factors influencing weight, number, flow rate, and size distribution of aerosol particles. Arch Int Pharmacodyn Ther 1964; 150:264–94.
107. Barton AD. Aerosolized detergents and mucolytic agents in the treatment of stable chronic obstructive pulmonary disease. Am Rev Respir Dis 1974; 110:104–10.
108. Limber CR, Reiser HG, Roettig LC, Curtis GM. Enzymatic lysis of respiratory secretions by aerosol trypsin. JAMA 1952; 149:816–21.
109. Feeley TW, DuMoulin GC, Hedley-Whyte J, Bushnell LS, Gilberg JP, Feingold DS. Aerosol polymyxin and pneumonia in seriously ill patients. N Engl J Med 1975; 293:471–5.
110. Kilburn KH. The innocuousness and possible therapeutic use of aerosol amphotericin-B. Am Rev Respir Dis 1959; 80:441–2.
111. Scott GH, Svdiskis RJ. Responses of mice immunized with influenza virus by aerosol and parenteral routes. Infect Immun 1976; 13:696–703.
112. Heves J, Catherall EJ. Aerosol chemotherapy of lung neoplasia. Nature 1974; 247:485–7.
113. Merrill EW, Graves DJ, Smith KA, Shannon DC, Kazemi H. Lecithin aerosols generated ultrasonically above 25° C. Science 1969; 164: 1167–8.
114. Hitchner SB, Reising G. Flock vaccination for Newcastle disease by atomization of the B_1 strain of virus. Proc Am Vet Med Assoc 1952; 89:258–264.
115. Middlebrook G. Immunological aspects of airborne infection: reactions to inhaled antigens. Bacteriol Rev 1961; 25:331–6.
116. Waldman RH, Mann JJ, Small PA. Immunization against influenza. JAMA 1969; 207:520–4.
117. Palmes ED,Wang C, Goldring RM, Altshuler B. Effect of depth of inhalation on aerosol persistence during breath holding. J Appl Physiol 1973; 34:356–60.
118. Pierce AK, Saltzman HA, eds. Conference on

the scientific basis of respiratory therapy. Am Rev Respir Dis 1974; 110:1–203.
119. Lertzman MM, Cherniack RM. Cardiopulmonary toxicity of propellants for aerosols. J Clin Pharmacol 1976; 114:1145–65.
120. Miller WF. Aerosol therapy in acute and chronic respiratory disease. Arch Intern Med 1973; 131:148–55.
121. Bau SK, Aspin N, Wood DE, Levison H. The measurement of fluid deposition in humans following mist tent therapy. Pediatrics 1971; 48:605–12.
122. Asmundsson T, Johnson RF, Kilburn KH, Goodrich JK. Efficiency of nebulizers for depositing saline in human lung. Am Rev Respir Dis 1973; 108:506–12.
123. Barker R, Levison H. Effects of ultrasonically nebulized distilled water on airway dynamics in children with cystic fibrosis and asthma. J Pediatr 1972; 80:396–400.
124. Chang N, Levison H, Cunningham K, Crozier DN, Grosett O. An evaluation of nightly mist tent therapy for patients with cystic fibrosis. Am Rev Respir Dis 1973; 107:672–5.
125. Aviado DM. Toxicity of aerosols. J Clin Pharmacol 1975; 28:86–104.
126. Belej MA, Aviado DM. Cardiopulmonary toxicity of propellants for aerosols. J Clin Pharmacol 1975; 15:105–15.
127. Silverglade A. Cardiac toxicity of aerosol propellants. JAMA 1972; 222:827–8.
128. Morrow PE. Some physical and physiological factors controlling the fate of inhaled substances. I. Health Phys 1960; 2:366–76.
129. Mercer TT. Aerosol technology in hazard evaluation. New York: Academic Press, 1973.
130. Fuchs NA. The mechanics of aerosols. Oxford: Pergamon Press, 1964.
131. Davies CN, ed. Aerosol science. New York: Academic Press, 1966.
132. Valberg PA, Brain JD, LeMott SR, Sneddon SL, Vinegar A. Dependence of aerosol deposition site on breathing pattern (abstract). Am Rev Respir Dis 1978; 117:262.
133. Dennis R, ed. Handbook on aerosols. Springfield: National Technical Information Service, U.S. Department of Commerce, 1976.
134. Mercer TT, Morrow PE, Staber W, eds. Assessment of airborne particles. Springfield, Ill.: Charles C Thomas, 1972.
135. Lippmann M. Respirable dust sampling. In: Air Sampling Instruments Committee. Air sampling instruments for evaluation of atmospheric contaminants, 5th ed. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1978: G1–G25.
136. Air Sampling Instruments Committee. Lippman M, Chrmn. Air sampling instruments for evaluation of atmospheric contaminants, 5th ed. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1978.
137. Cartwright J. Particle shape factors. Ann Occup Hyg 1962; 5:163–71.
138. Tillery M. A concentric aerosol spectrometer. Am Ind Hyg Assoc J 1974; 35:62–74.
139. Mazumder MK, Kirsch KJ. A single particle aerodynamic relaxation time analyzer. Rev Sci Instrum 1977; 48:622–4.
140. Tillery MI, Wood GO, Ettinger HJ. Generation and characterization of aerosols and vapors for inhalation experiments. Environ Health Perspect 1976; 16:25–40.
141. Lang RJ. Ultrasonic atomization of liquids. J Acoust Soc Am 1962; 34:6–9.
142. Brain JD, Valberg PA, Sorokin SP, Hinds WC. An iron oxide aerosol suitable for animal exposures. Environ Res 1974; 7:13–26.
143. Valberg PA, Brain JD. Generation and use of three types of iron-oxide aerosol. Am Rev Respir Dis, 1979; 120:1013–1024.
144. Raabe OG. The generation of aerosols of fine particles. In: Liu BYH, ed. Fine particles: aerosol generation, measurement, sampling and analysis. New York: Academic Press, 1976:57–110.
145. Strom L. The generation of monodisperse aerosols by means of disintegrated jet of liquid. Rev Sci Instrum 1969; 40:778–82.
146. Berglund RN, Liu BYH. Generation of monodisperse aerosol standards. Environ Sci Technol 1973; 7:147–53.
147. Kotrappa P, Moss OR. Production of relatively monodisperse aerosols for inhalation experiments by aerosol centrifugation. Health Phys 1971; 21:531–5.
148. Bynum LJ, Pierce AK. Pulmonary aspiration of gastric contents. Am Rev Respir Dis 1976; 114:1129–36.
149. Ribaudo CA, Grace WJ. Pulmonary aspiration. Am J Med 1971; 50:510–20.
150. Bartlett JG, Gorbach SL. The triple threat of aspiration pneumonia. Chest 1975; 68:560–6.
151. Amberson JB. Aspiration bronchopneumonia. Int Anesthesiol Clin 1937; 3:126–8.
152. Smith DT. Experimental aspiratory abscess. Arch Surg 1927; 14:231–6.
153. Bartlett JG, Finegold SM. Anaerobic pleuropulmonary infections. Medicine (Baltimore) 1972; 51:413–50.
154. Bartlett JG, Gorbach SL, Finegold SM. The bacteriology of aspiration pneumonia. Am J Med 1974; 56:202–7.
155. Brain JD, Knudson DE, Sorokin SP, Davis MA. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ Res 1976; 11:13–33.
156. Findeisen W. Uber das Absetzen kleiner, in der Luft suspendierten Teilchen in der menschlichen Lunge bei der Atmung. Pfluegers Arch 1935; 236:367–79.

157. Landahl HD. On the removal of airborne droplets by the human respiratory tract: I. The lung. Bull Math Biophys 1950; 12:43–56.
158. Beeckmans JM. The deposition of aerosols in the respiratory tract. I. Mathematical analysis and comparison with experimental data. Can J Physiol Pharmacol 1965; 43:157–72.
159. Altshuler B, Palmes ED, Nelson N. Regional aerosol deposition in the human respiratory tract. In: Davies CN, ed. Inhaled particles and vapours II. New York: Pergamon Press, 1966: 323–35.
160. Altshuler B. Calculation of regional deposition of aerosol in the respiratory tract. Bull Math Biophys 1959; 21:257–70.
161. Goodwin PN, Rao DV. An introduction to the physics of nuclear medicine. Springfield, Ill.: Charles C Thomas, 1977.
162. Freeman LM, Johnson PM, eds. Clinical scintillation imaging. New York: Grune and Stratton, 1975.
163. Cuddihy RG, Boecker BB. Controlled administration of respiratory tract burdens of inhaled radioactive aerosols in beagle dogs. Toxicol Appl Pharmacol 1973; 25:597–605.
164. Morsy SM, Werner F, Stahlhofen W, Pohlit W. A detector of adjustable response for the study of lung clearance. Health Phys 1977; 32:243–51.
165. Parker RP, Smith PHS, Taylor DM. Basic science of nuclear medicine. New York: Churchill Livingstone, 1978.
166. Herz RH. The photographic action of ionizing radiations. New York: Wiley-Interscience, 1969.
167. Rogers AW. Techniques of autoradiography, 3rd ed. New York: Elsevier/North Holland, 1978.
168. Cember H. Empirical establishment of cancer-associated dose to the lung from ^{144}Ce. Health Phys 1964; 10:1177–80.
169. Brain JD, Valberg PA, Sneddon SL, LeMott SR. Techniques for quantifying patterns of aerosol deposition (abstract). Fed Proc 1978; 37:927.
170. McCallum RI, Day MH, Underhill J, Aird EGA. Measurement of antimony oxide dust in human lungs in vivo by X-ray spectrophotometry. In: Walton WH, ed. Inhaled Particles III. Old Woking: Unwin Bros, 1971:611–20.
171. Friedman M, Stott FD, Poole DO, Dougherty R, Chapman GA, Watson H, Sackner MA. A new roentgenographic method for estimating mucous velocity in airways. Am Rev Respir Dis 1977; 115:67–72.
172. Cohen D. Ferromagnetic contamination in the lungs and other organs of the human body. Science 1973; 180:745–8.
173. Cohen D. Measurement of the magnetic field produced by the human heart, brain and lungs. IEEE Trans Magnet 1975; MAG-11 (No. 2): 694.
174. Cohen D. Report of the low-field group: the magnetic field of the lung. Springfield, Va.: National Technical Information Service, publication no. MIT/FBNML-78/1, 1978.
175. Kalliomaki PL, Karp PK, Katila T, Makipaw P, Jaar P, Tossavainan A. Magnetic measurements of pulmonary contamination. Scand J Work Environ Health 1976; 4:232–9.
176. Cohen D, Arai SF, Brain JD. Smoking impairs long-term dust clearance from the lungs. Science 1979; 204:514–17.
177. Watson AY, Brain JD. Uptake of iron oxide aerosols by mouse airway epithelium. Lab Invest 1979; 40:450–9.
178. Sorokin SP, Brain JD. Pathways of clearance in mouse lungs exposed to iron oxide aerosols. Anat Rec 1975; 181:581–625.
179. Landahl HD. Particle removal by the respiratory system. Bull Math Biophys 1963; 25:29–39.
180. Horsfield K, Dart G, Olson DE, Filley GF, Cumming G. Models of the human bronchial tree. J Appl Physiol 1971; 31:207–17.
181. Dautrebande L, Walkenhorst W. Deposition of microaerosols in human lung with special reference to the alveolar spaces. Health Phys 1964; 10:981–93.
182. Morrow PE. Aerosol characterization and deposition. Am Rev Respir Dis 1974; 110:88–99.
183. Lippmann M, Albert RE. The effect of particle size on the regional deposition of inhaled aerosols in the human respiratory tract. Am Ind Hyg Assoc J 1969; 30:257–75.
184. Giacomelli-Maltoni G, Melandri C, Prodi V, Tarroni G. Deposition efficiency of monodisperse particles in human respiratory tract. Am Ind Hyg Assoc J 1972; 33:603–10.
185. Foord N, Black A, Walsh M. Pulmonary deposition of inhaled particles with diameters in the range 2.5 to 7.5 m. In: Walton WH, ed. Inhaled Particles IV. Part 1. New York: Pergamon Press, 1977:137–50.
186. Davies CN, Lever MJ, Rothenberg SJ. Experimental studies of the deposition of particles in the human lungs. In: Walton WH, ed. Inhaled particles IV, New York: Pergamon Press, 1977:151–62.
187. Heyder J, Gebhart J, Heigver G, Roth C, Stahlhofen W. Experimental studies of the total deposition of aerosol particles in the human respiratory tract. J Aerosol Sci 1973; 4:191–208.
188. Schlesinger RB, Lippmann M. Selective particle deposition and bronchogenic carcinoma. Environ Res 1978; 15:424–31.
189. Morgan WKC, Seaton A. Occupational lung diseases. Philadelphia: WB Saunders, 1975.
190. Stokinger HE, Steadman LT, Wilson HB, Sylvester GE, Dziuba S, LaBelle CW. Lobar deposition and retention of inhaled insoluble par-

ticulates. Arch Ind Hyg Occup Med 1951; 4: 346–53.

191. Raabe OG, Yeh H-C, Newton GJ, Phalen RF, Velasquez DJ. Deposition of inhaled monodisperse aerosols in small rodents. In: Walton WH, ed. Inhaled particles IV. New York: Pergamon Press, 1977:3–22.
192. Thomas RL, Raabe OG. Regional deposition of inhaled ^{137}Cs-labeled monodisperse and polydisperse aluminosilicate aerosols in Syrian hamsters. Am Ind Hyg Assoc J 1978; 39:1009–18.
193. Yu CP, Taulbee DB. A theory of predicting respiratory tract deposition of inhaled particles in man. In: Walton WH, ed. Inhaled particles IV. Part 1. New York: Pergamon Press 1977: 35–48.
193a. Weibel E.R. Morphometry of the human lung. Springer, Berlin-Heidelberg, 1963.
194. Heyder J, Rudolf G. Deposition of aerosol particles in the human nose. In: Walton WH, ed. Inhaled particles IV. Part 1. New York: Pergamon Press, 1977:107–26.
195. Bell KA. Aerosol deposition in models of a human lung bifurcation. PhD Thesis, California Institute of Technology, 1974.
196. Bell KA, Friedlander SK. Aerosol deposition in models of a human lung bifurcation. Staub Reinhalt Luft 1973; 33:183–7.
197. Schlesinger RB, Lippmann M. Particle deposition in the trachea: *in vivo* and in hollow casts. Thorax 1976; 31:678–84.
198. Schlesinger RB, Lippmann M. Particle deposition in casts of the human upper tracheobronchial tree. Am Ind Hyg Assoc J 1972; 33: 237–51.
199. Shaw DT, Rajendran N, Liao NS. Theoretical modeling of fine-particle deposition in 3-dimensional bronchial bifurcations. Am Ind Hyg Assoc J 1978; 39:195–201.
200. Harris RL, Timbrell V. The influence of fibre shape in lung deposition: mathematical estimates. In: Walton WH, ed. Inhaled particles IV. New York: Pergamon Press, 1977:75–92.
201. Morgan A, Evans JC, Holmes A. Deposition and clearance of inhaled fibrous minerals in the rat: studies using radioactive tracer techniques. In: Walton WH, ed. Inhaled particles IV. Part 1. New York: Pergamon Press, 1977: 259–74.
202. Cinkotai FF. The behaviour of sodium chloride particles in moist air. J Aerosol Sci 1971; 2:325–9.
203. Sinclair DR, Countess J, Hoopes GS. The effect of relative humidity on the size of atmospheric aerosol particles. Atmos Environ 1974; 8:111–17.
204. Ferron GA. The size of soluble aerosol particles as a function of the humidity of the air: application to the human respiratory tract. J Aerosol Sci 1977; 8:251–67.
205. Longley MY. Pulmonary deposition of dust as affected by electric charges on the body. Am Ind Hyg Assoc J 1960; 21:187–94.
206. Brock JR, Marlow WH. Charged aerosol particles and air pollution. Environ Letters 1975; 10:53–67.
207. Fry FA. Charge distribution on polystyrene aerosols and deposition in the human nose. J Aerosol Sci 1970; 1:135–46.
208. Melandri C, Prodi V, Tarroni G, Formignani M, DeZiacomo T, Bompane GF, Maestri G. On the deposition of unipolarly charged particles in the human respiratory tract. In: Walton WH, ed. Inhaled particles IV. New York: Pergamon Press, 1977:193–201.
209. Chan TL, Lippmann M, Cohen VR, Schlesinger RB. Effect of electrostatic charges on particle deposition in a hollow cast of the human larynx-tracheobronchial tree. J Aerosol Sci 1978; 9:463–8.
210. Dennis WL. The effect of breathing rate on the deposition of particles in the human respiratory system. In: Walton WH, ed. Inhaled Particles III. Surrey, England: Unwin Brothers, 1971:91–102.
211. Bouhuys A, Proctor DF, Mead J. Kinetic aspects of singing. J Appl Physiol 1966; 21:2–10.
212. Bunn JC, Mead J. Control of ventilation during speech. J Appl Physiol 1971; 31:870–2.
213. Muir DCF, Davies CN. The deposition of 0.5 μm diameter aerosols in the lungs of man. Ann Occup Hyg 1967; 10:161–74.
214. Davies CN, Heyder J, Ramu MCS. Breathing of half-micron aerosols. I. Experimental. J Appl Physiol 1972; 32:591–600.
215. Davies CN. Breathing of half-micron aerosols. II. Interpretation of experimental results. J Appl Physiol 1972; 32:601–11.
216. Pavia D, Thomson M, Shannon HS. Aerosol inhalation and depth of deposition in the human lung. Arch Environ Health 1977; 32: 131–7.
217. Taulbee DB, Yu CP, Heyder J. Aerosol transport in the human lung from analysis of single breaths. J Appl Physiol 1978; 44:803–12.
218. Foord N, Black A, Walsh M. Regional deposition of 2.5–7.5 μm diameter inhaled particles in healthy male non-smokers. J Aerosol Sci 1978; 9:343–57.
219. Hounam RF, Morgan A. Particle deposition. In: Brain JD, Proctor DF, Reid LM, eds. Respiratory defense mechanisms. Part I. New York: Marcel Dekker, 1977:125–56.
220. Brain JD. The uptake of inhaled gases by the nose. Ann Otol Rhinol Laryngol 1970; 79: 529–39.
221. Pattle RE. The retention of gases and particles

in the human nose. In: Davies CN, ed. Inhaled particles and vapors. Oxford: Pergamon Press, 1961:302–9.
222. Hounam RF, Black A, Walsh M. Deposition of aerosol particles in the nasopharyngeal region of the human respiratory tract. Nature 1969; 221:1254–5.
223. Lippmann M. Deposition and clearance of inhaled particles in the human nose. Ann Otol Rhinol Laryngol 1970; 79:519–28.
224. Martens A, Jacobi W. Die *in vivo* Bestimmung der Aerosolteilchendeposition im Atemtrakt bei Mund—bzw. Nasenatmung. In: Aerosole in Physik, Medizin und Technik Bad Soden, W. Germany: Gesellschaft fur Aerosolforschung 1974:117–21.
225. Proctor DF. The upper respiratory tract and the ambient air. Clin Notes Respir Dis 1974; 12:2–10.
226. Williams NJ. Potash and perforation of the nasal system. N J Occup Med 1974; 16:383–7.
227. Andersen HC, Andersen I, Solgaard J. Nasal cancers, symptoms and upper airway function in woodworkers. Br J Ind Med 1977; 34:201–7.
228. Acheson ED. Nasal cancer in the furniture and boot and shoe manufacturing industries. Prev Med 1976; 5:295–315.
229. Acheson ED, Cowdell RH, Hadfield E, Macbeth RG. Nasal cancer in woodworkers in the furniture industry. Br Med J 1968; 2:587–96.
230. Mosbech J, Acheson GD. Nasal cancer in furniture-makers in Denmark. Dan Med Bull 1971; 18:34–5.
231. Stell PM, McGill T. Asbestos and cancer of the head and neck. Lancet 1973; 1:678.
232. Doll R, Morgan LG, Seizer FE. Cancers of the lung and nasal sinuses in nickel workers. Br J Cancer 1970; 24:623–32.
233. Virtue JA. The relationship between the refining of nickel and cancer of the nasal cavity. Can J Otolaryngol 1972; 1:37–42.
234. Heuper WC. Occupational and environmental cancers of the respiratory system. New York: Springer Verlag, 1966.
235. Hems G. The risk of bone cancer in man from internally deposited radiation. Br J Radiol 1967; 40:506–10.
236. Palm PE, McNerney JM, Hatch T. Respiratory dust retention in small animals. Arch Ind Health 1956; 13:355–65.
237. Friedlander SK. Particle deposition by diffusion in the lower lung: application of dimensional analysis. Am Ind Hyg Assoc J 1964; 25: 37–42.
238. Kliment V. Similarity and dimensional analysis, evaluation of aerosol deposition in the lungs of laboratory animals and man. Folia Morphol (Praha) 1973; 21:59–64.
239. Stauffer D. Scaling theory for aerosol deposition in the lungs of different mammals. J Aerosol Sci 1975; 6:223–5.
240. McMahon TA, Brain JD, LeMott SR. Species differences in aerosol deposition. In: Walton WH, ed. Inhaled particles IV. Oxford: Pergamon Press, 1977:23–33.
241. Stahl WR. Scaling of respiratory variables in mammals. J Appl Physiol 1967; 22:453–60.
242. McMahon TA. Size and shape in biology. Science 1973; 179:1201–4.
243. Thomas RG. an interspecies model for retention of inhaled particles. In: Mercer TT, Morrow PE, Stober W, eds. Assessment of airborne particles, Springfield, Ill.: Charles C. Thomas 1972:405–55.
244. Lippmann M, Albert RE, Peterson HT Jr. The regional deposition of inhaled aerosols in man. In: Walton WH, ed. Inhaled particles III. Surrey, England: Unwin Brothers, 1971; 105–20.
245. Goldberg IS, Lourenço RV. Deposition of aerosols in pulmonary disease. Arch Intern Med 1973; 131:88–91.
246. Lourenço RV, Loddenkemper R, Cargon RW. Patterns of distribution and clearance of aerosols in patients with bronchiectasis. Am Rev Respir Dis 1972; 106:857–66.
247. Jakab GJ, Green GM. Effects of pneumonia on intrapulmonary distribution of inhaled particles. Am Rev Respir Dis 1973; 107:675–8.
248. Pavia D, Thomson ML, Clarke SW, Shannon HS. Effect of lung function and mode of inhalation on penetration of aerosol into the human lung. Thorax 1977; 32:144–97.
249. Punte CL, Owens EJ, Krackow EH, Cooper PL. Influence of physical activity on the toxicity of aerosols and vapors. Arch Ind Health 1958; 17:34–7.
250. Kendrick J, Rubin IB, Creasia DA, Maddox WL, Guerin MR, Nettesheim P. Respiratory tract deposition of smoke particles using a nasal bypass device. Arch Environ Health 1976; 31:131–5.
251. Sneddon SL, Brain JD, LeMott SR, Valberg PA. Factors influencing aerosol deposition in small animals (abstract). Fed Proc 1978; 37: 927.
252. Hoffman RA, Bililngham J. Effect of altered G levels on deposition of particulates in the human respiratory tract. J Appl Physiol 1975; 38:955–60.
253. Muir DCF. Deposition and clearance of inhaled particles. In: Muir DCF, ed. Clinical aspects of inhaled particles. Philadelphia: Davis 1972:1–20.
254. Sikov MR, Mahlum DD. Influence of age and physicochemical form on the effects of ^{239}Pu on the skeleton of the rat. In: Jee WSS, ed. The health effects of plutonium and radium. Salt Lake City,Utah: JW Press, 1976:33–47.